THE LYMPHOCYTE
and
LYMPHOCYTIC TISSUE

INTERNATIONAL ACADEMY OF PATHOLOGY

MONOGRAPHS IN PATHOLOGY

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No 1 The Lymphocyte and Lymphocytic Tissue
No 2 The Adrenal Cortex

Succeeding volumes to be announced

INTERNATIONAL ACADEMY OF PATHOLOGY MONOGRAPH

THE LYMPHOCYTE

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LYMPHOCYTIC TISSUE

by 21 authors

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CONTENTS

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FOREWORD

Since 1953 the International Academy of Pathology has held annual courses on the pathologic anatomy and pathologic physiology of various organ systems Past subjects have included lung, liver, heart, erythrocyte, skin, and bone. The program for each course has been developed by Dr. F. K. Mostofi, Secretary-Treasurer of the Academy, with the assistance of the respective moderators These courses have proved both valuable and increasingly popular For those attending, the material presented by outstanding authorities has been an excellent review of important aspects of normal and abnormal structure, function and development

In accordance with the expanding educational activities of the Academy, its Council has decided to sponsor a series of books, Monographs in Pathology, to publish the material from its courses, which bring together in organized fashion essential information not otherwise so conveniently available This will give access to the course material to those unable to attend and will provide a lasting source of reference for those who do attend. The many requests for such a publication give the Council confidence that it and succeeding volumes will be useful

This initial volume, *The Lymphocyte and Lymphocytic Tissue,* contains the material from the course moderated by Dr John W Rebuck at the 1959 Academy meeting in Boston The second monograph will contain the proceedings of the 1960 course to be held in Memphis, Tennessee, on "Pathologic Physiology and Pathology of the Adrenal Cortex," with Dr Henry Moon as moderator

These monographs will contribute to the development of the reference literature in the field of pathology Manuscripts selected for publication in this series will meet the highest standards of scientific authenticity and educational value While the first volumes of the Monographs in Pathology will consist of the courses mentioned, the Council of the Academy hopes also to develop other types of monographs by individual authors or in symposium

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PREFACE

Far-reaching advances in our knowledge of the lymphocyte have merited this present evaluation First, Downey and his students supplemented information gained by histologic techniques with the advanced cytologic information to be obtained by imprints of lymphocytic tissues. Next Dougherty and White and the Harrises demonstrated antibody formation by lymphocytes Soon thereafter study of human skin windows revealed that lymphocytes were the major mobile source of macrophages in the inflammatory exudates of man. Integrated with all these findings was the realization afforded by the work of Ottesen and Hamilton of an exceptionally long life span possessed by lymphocytes, a life span now measured in weeks and months instead of in hours or days The viral etiology of neoplasms of the lymphocyte is now well documented by the experiments of Gross and Schwartz Added to these must be the ultrastructural descriptions of Bessis and Low, the ascertainment by Dougherty and his associates of an exquisite sensitivity of normal lymphocytes to certain steroids notwithstanding the refractoriness of their leukemic counterparts, the somewhat similar response to irradiation pointed out by Schrek—all are facets of the newer knowledge of the lymphocytes presented in the following pages.

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Detroit

form which will contribute to our knowledge of pathology, to the training of pathologists, and to the development of the discipline. Comments and suggestions from readers of this volume will be most welcome.

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CHAPTER 1

LYMPHOCYTES: ORIGIN, STRUCTURE, AND INTERRELATIONSHIPS

R. DOROTHY SUNDBERG

In a previous review (1955)[1] on lymphocytes and plasma cells, I summarized much of the current literature pertaining to the problems of origin, development, interrelationships, and possible functions of these two cell types Currently my task is similar, but since the interpretation of the results of modern labeling techniques[2-6] has suggested that lymphocytes are reutilized in the formation of lymphocytes and that circulating blood cells (lymphocytes of some type or monocytes)[6] are capable of division, I plan to present illustrations that may prove useful in respect to both of these facets of the problem

In this regard, the morphologic features characterizing the cells commonly found in the germinal centers will be reconsidered, and the reasons for acknowledging somewhat different cell tyes within germinal centers will be re-emphasized

LYMPHOCYTOGENESIS

The origin and development of lymphocytes are difficult to study because lymphatic tissue consists of a variable mixture of reticular connective tissue with its vascular supply and perivascular cells, lymphocytes, plasma cells, histiocytes, macrophages, and a few cells that might be classified as monocytes. In normal lymph nodes mitoses may be found in any part of the node, but they are most numerous in the germinal centers of Flemming where one finds macrophages with stainable bodies[7] as well as developing lymphocytes and plasma cells Considered on the basis of the bulk of lymphatic tissue, mitoses

are relatively sparse, and thus an appreciation of which cell types divide is difficult to attain. The exact nature of the dividing cell is seldom indisputable; one judges the cell type for the most part on the basis of the size of the mitotic figure and the nature of the cytoplasm, both of which are altered by *the process of division.*

Studies of section and imprint material from human lymph nodes have resulted in the conclusion that there are many transitional stages[8] of development from reticular cells to lymphocytes; mitoses occur in all of the stages *of development, but they appear to be exceedingly rare in cells comparable to* the small lymphocytes of the blood. Mitoses have been said to be most numerous in the medium-sized and large lymphocytes of lymph nodes[9-12] but it should be realized that these cells only rarely are found in the circulating blood. *In infants and in laboratory animals, immature lymphocytes and retic*ular lymphocytes are regarded as possible constituents of the blood.

A previous concept of lymphocytogenesis,[1, 8] with which the late Dr. Hal Downey agreed, can be expressed in the following schema

Sections of lymphatic tissue
Primitive reticular cells
(mitoses)
↓
Giant and large lymphocytes
(hemocytoblasts)
(mitoses)
↓
Medium-sized lymphocytes
(mitoses)
↓
Small lymphocytes
(mitoses rare)

Imprints of lymphatic tissue
Primitive reticular cells
(mitoses)
↓
Hematopoietic reticular cells
(reticular blast forms)
(mitoses)
↓
Reticular lymphocytes
Immature lymphocytes
(prolymphocyts)
(mitoses)
↓
Large medium sized, and small lymphocytes
(mitoses rare)

The cells that *function* as lymphoblasts of the node have been considered *as progeny of reticular cells in order to explain one facet of the process of* lymphocytogenesis,[1, 7, 14] but the fact that lymphocytes may enlarge and become indistinguishable from histiocytes, macrophages, and reticular cells has been demonstrated by many investigators.[14-16] This reverse (?) transformation of lymphocytes to large basophilic cells resembling hematopoietic reticular cells[17] has been acknowledged,[13] and the fact of ultimate transformation of these cells to histiocytes, macrophages, epithelioid cells, giant cells, and fibroblasts has become part of the general morphologic understanding of in-

flammatory reactions. The fact that some type of blood cell (lymphocyte?) can even become an epithelial like cell in tissue culture has been described and illustrated by Berman.[18]

An intriguing and plausible concept is that of Trowell[19, 20] who suggested that reticulum cells containing phagocytosed lymphocytes may develop to the more rounded precursors of lymphocytes while still containing visible remains of ingested lymphocytes. He felt this concept would explain the life-span data of Hamilton[2, 5] and others[21-25] and offers this morphologic explanation of reutilization of lymphocytes as well as of lymphocytogenesis. With this in mind, he is prepared to dispense with the primitive or undifferentiated reticulum cell and to propose instead that there is but one type of reticulum cell.

This concept seems refreshing and acceptable, but whether or not it covers all situations is difficult to say. Yoffey[26] is not prepared to endorse this wholeheartedly because phagocytosis, lymphocytolysis, and lymphocytogenesis are unequally represented in germinal centers and other areas of lymphocytogenesis, and the association of macrophages and mitotic figures is not clear-cut.

Here it should be emphasized that for many years Downey stressed that reticular cells had diverse functions (phagocytosis, storage, support, and lining as well as hematopoiesis), but he also considered the cells sufficiently versatile to do more than one thing at once. Examples of this include: the ability of the Gaucher cell to phagocytose erythrocytes and contain hemosiderin as well as to store kerasin[27] and the ability of the hematopoietic reticular cells of leukemic reticuloendotheliosis to be actively phagocytic.[28, 29] Downey's illustrations of the "histiocytes" of subacute bacterial endocarditis show the transformation of this type of cell to a monocyte.[29] One of the reticular cells (Fig. 27 in Reference 29) contains a dark body which might well be nuclear debris.

I have spent many years wondering what the macrophages of the bone marrow do with the many things they ingest *in toto* (erythrocytes, normoblasts, neutrophils, lymphocytes, platelets, and occasionally other cell types as well as iron, melanin, and unidentified stainable materials). My preoccupation with storage iron[10, 11] and melanin[30, 44] has kept me from investigating the other cytoplasmic contents of these macrophages sufficiently thoroughly to draw definite conclusions concerning disposition of these cytoplasmic contents. (In the case of reticular cells containing phagocytosed erythrocytes or iron, I have assumed that the reticular cells were processing the red cell or the iron for reutilization. In the case of reticular cells containing numerous neutrophils and their autolyzed remains—in the absence of agranulocytosis—I have tried unsuccessfully to correlate this with hyperuricemia and have

wished to spend time investigating methods of staining these autolyzed remains for the degradation products of nucleic acid.)

I did not concern myself with the stainable Körper of reticulum cells in my previous studies not because they were not interesting but rather because I felt I had seen morphologic evidence that seemingly nonphagocytic reticular cells rounded up to produce blast forms. My illustrations attest the difficulty I had, for our undifferentiated reticular cell (Fig. 7 in Reference 13) from the lymph node of a rabbit *does* contain phagocytosed lysed material in its cytoplasm (upper left corner, Fig. 7 in Reference 13) and one of my later cells

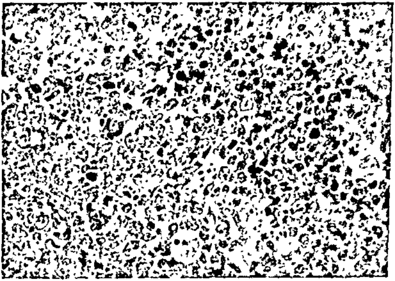


FIG 1-1 Section of germinal center of hyperplastic lymph node Note reticular cells with stainable bodies Note also numerous mitotic figures (× 410)

(human lymph nodes) (Fig. 2 in Reference 8) was admittedly a "potential phagocyte" Even the undifferentiated reticular cell shows an intracytoplasmic, oval, white inclusion (vacuole?) in the pseudopod-like process extending to the right from the nucleus (Fig 1 in Reference 8)

With Trowell's hypothesis in mind, I have reviewed some of the material I used in the previous studies[8, 13] and have confirmed the fact that morphologically there does appear to be a complete series of lymphocyte-eating and lymphocyte-digesting cells, the last cells of the series may show a remarkable resemblance to cells I believe to be capable of functioning as precursors of lymphocytes (hematopoietic reticular cells and reticular lymphocytes) This series of cells is illustrated in Figures 1-1 through 1-8

Of particular interest to me have been the types of cells shown in Figures 1-9 through 1-12. Many of these reticular cells have nuclear patterns coarser and more definitive in morphology than those seen in the usual blast forms (hematopoietic reticular cells) of leukemic reticuloendotheliosis. The nuclei are comparable to the nuclei of histiocytes and macrophages, and the cytoplasm often has no clear delimiting membrane. These reticular cells are comparable to the hemohistioblasts of Ferrata[11] and are often confused with cells that are actually damaged cells (with ruptured nuclear membranes and

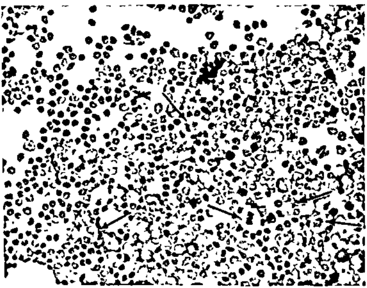


FIG 1 2 Imprint from part of germinal center of same lymph node shown in Fig 1 1. Note 5 mitotic figures (*arrows*) and reticular cell with stainable bodies (engulfed lymphocytes in various stages of digestion) designated by X Note plasma cell above phagocyte (× 220)

frayed cytoplasm), but since the former undergo mitosis even without apparent intact cytoplasmic boundaries, I have maintained that they are not just damaged cells but rather are probably the cells that are most comparable to the fixed reticular cells This same cell type can be found in small numbers in films of nonleukemic bone marrow where again I have thought it was probably one of the fixed reticular cells that had torn loose from its syncytium

If one combines the morphologic evidence gleaned from examination of the transitional forms from phagocytic reticular cells (Figs 1-2 through 1-6) to reticular cells in which the evidence of previous phagocytosis is obscure (Figs.

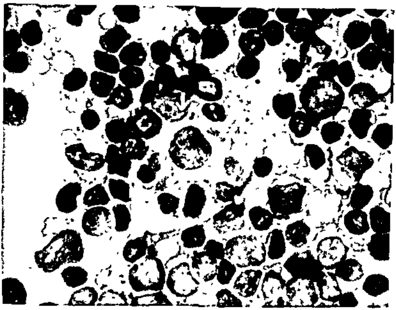


FIG 1-3 One large reticular cell contains 5 obvious stages in the degeneration and digestion of lymphocytes This imprint is near same area shown in Fig 1-2 The arrow points to a white line, the edge of the cytoplasm of the reticular cell, which is covering the lymphocyte A similar edge is seen over the lymphocyte at the 9 o'clock position (× 800)

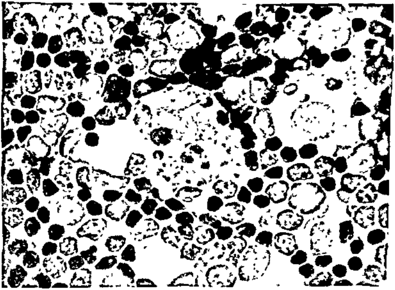


FIG 1 4 Two large reticular cells from area near or in same germinal center shown in Fig 1 2 Note various stages of degeneration or digestion of engulfed cells (× 800)

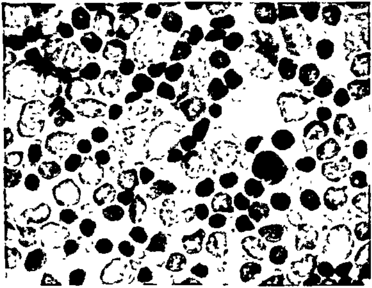


Fig. 1-5 Note reticular cell containing remains of lymphocytes and reticular lymphocyte near center of field Note immature lymphocyte at lower right hand corner Imprint is near area shown in Fig 1-2 (× 800)

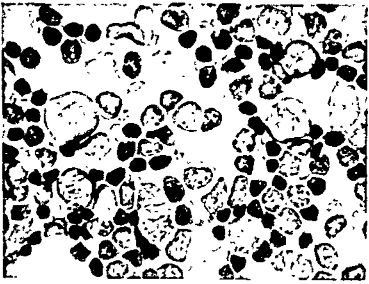


Fig 1-6 Note from left to right hematopoietic reticular cell reticular cell containing engulfed lymphocyte reticular lymphocyte, and reticular cell containing stainable body Note also the mitotic figure above central reticular cell Imprint is near area shown in Fig 1-2 (× 800)

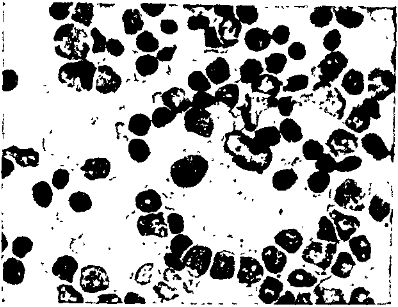


FIG 1-7 Large reticular cell contains flocculent masses in cytoplasm (cellular debris) Most of the peripheral portion of the cell is surrounded by lymphocytes. An immature lymphocyte is embedded in the cytoplasm just to the right of the nucleus Imprint is near area shown in Fig 1-2. (× 800)



FIG 1-8 Note 2 similar reticular cells which still show evidence of previous phagocytosis (Compare these cells with that in Fig 1 7 and those reticular cells with coarse nuclear pattern in Figs 1-9 through 1 12) In the cells shown above, the cytoplasm ("ectoplasmic portion") extends out much farther from the nucleus than is apparent at first glance Imprint is near area shown in Fig 1-2 (× 800)

1-7, 1-8) to the "hematopoietic reticular cells" in the blood in leukemic reticuloendotheliosis (Figs. 1-9 through 1-12), it is apparent that Trowell's hypothesis[19, 20] can be supported by material seen in dry films as well as in sections. I failed to recognize this evidence prior to reading his papers. In 1947 I provided illustrations (Figs. 1 and 2 of Reference 8) of cells which are obviously "histiocytic" to emphasize the fact that the hematopoietic reticular cell in Figure 1-3 had a reticular nucleus, but I did not recognize the significance of my illustrations.



FIG. 1-9. Blood film from leukemic reticuloendotheliosis. Note the nucleus of reticular cell in center, the cytoplasm of this cell has indistinct peripheral boundaries. (This reticular cell is remarkably similar to the phagocytic reticular cells of the lymph node imprints.) The reticular cell to the left and below it is virtually identical with it, but the cytoplasm is hyaline, almost colorless, and distinctly bounded. This is a hematopoietic reticular cell as is the cell to the right of it, but the latter shows a more leptochromatic nuclear pattern and more cytoplasmic basophilia. (× 800)

Histochemical analyses of the phagocytic cells utilizing desoxyribonuclease and ribonuclease should give some insight into the nature of the cytoplasmic contents of many varying colors, which contents seem to have been confused with the cell's own cytoplasmic constituents. Utilizing ribonuclease, we have removed the basophilic component from cells of the bone marrow, including osteoblasts,[31,35] and have demonstrated that subsequent to this procedure, alkaline phosphatase is still present in osteoblasts. Using the Prussian blue reaction as a "counterstain," we have "removed" reticulation from reticulocytes and normoblasts and have found that many flocculent blue staining masses and granules contained in macrophages of the marrow and the lymph nodes have disappeared. If counterstaining with the Prussian blue reagent is

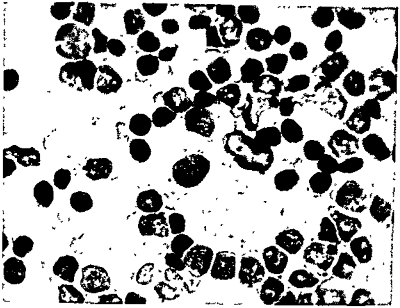


Fig 1-7. Large reticular cell contains flocculent masses in cytoplasm (cellular debris) Most of the peripheral portion of the cell is surrounded by lymphocytes An immature lymphocyte is embedded in the cytoplasm just to the right of the nucleus Imprint is near area shown in Fig 1 2 (× 800)

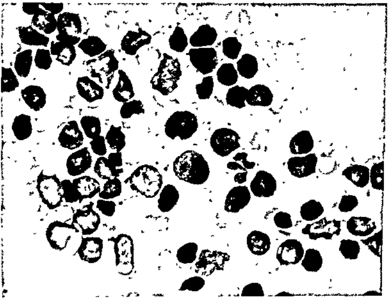


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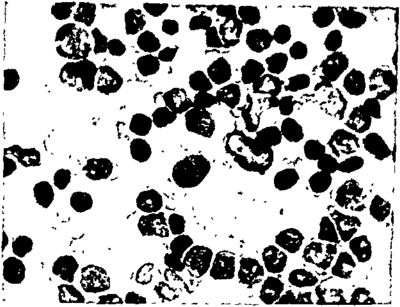


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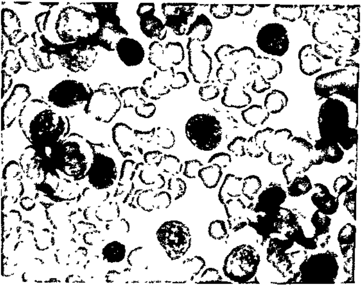


FIG 1-10 Note various types of reticular cells in blood film from leukemic reticuloendotheliosis The 4 cells at the left show some evidence of differentiation from lymphocytes. (× 800)

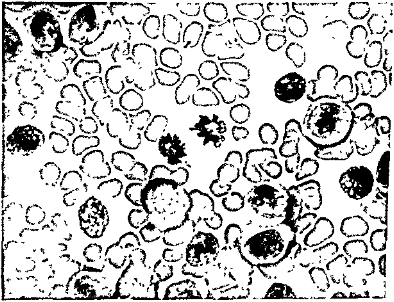


FIG 1-11 Blood film from leukemic reticuloendotheliosis Note mitotic figure The cytoplasm is comparable to that seen in the central reticular cell of Fig 1-9 and to the seemingly ragged and damaged cytoplasm of 5 of the reticular cells in this figure (× 800)

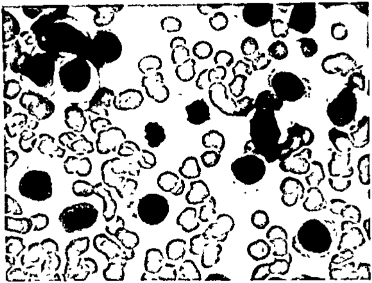


Fig. 1-12 Blood film from leukemic reticuloendotheliosis Note mitotic figure In triangular extensions of cytoplasm extruding up and to the left of each aster are circular masses of the endoplasmic portion of the cytoplasm Note various morphologic types of reticular cells in the surrounding field (× 800)

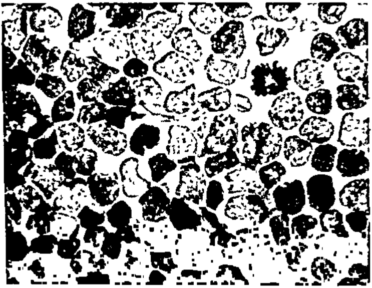


Fig. 1-13 Lymph node imprint from patient with lymphoblastic lymphoma Note lymphoblasts, many of which retain some nuclear characteristics of reticular cells The blood and marrow were negative at the time of this biopsy but the character of lymphoblasts indicated that diffuse leukemic involvement would occur (× 800)

sufficiently brief, nuclei are generally not robbed of their DNA because nuclear remnants are visible in erythrocytes as well as in macrophages.[30]

Because I have recently been convinced by the beautiful demonstration of Bessis[36] of the manner in which islands consisting of a reticular cell surrounded by normoblasts function, I should like to explain this briefly and draw a possible analogy for future investigation. Bessis has shown that the ring of normoblasts surrounding reticular cells of the marrow is more than a

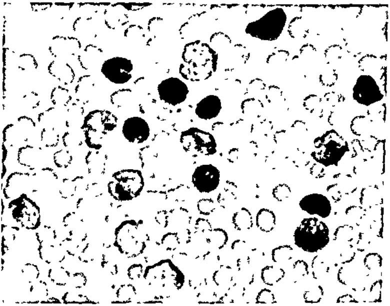


FIG 1-14. Blood from same patient from whom lymph node biopsy was obtained One reticular blastic form like those in the node is seen at the 12 o'clock position The next smaller cells are the classic lymphoblasts of acute and subacute lymphatic leukemia The hyperchromatic small cells are probably small or shrunken reticular cells, they are not normal small lymphocytes This blood was obtained late in the course of the disease when the marrow was also diffusely involved (× 800)

chance association We have known that reticular cells engulf erythrocytes and that within the reticular cells the erythrocytes lose the red-orange color of hemoglobin and ultimately leave white round remnants of themselves in that phagocytic cell The cytoplasm of the macrophage surrounding these phagocytosed erythrocytes may be intensely positive by the Prussian blue reaction. We have also known that the normoblasts surrounding this reticular cell may contain stainable particulate iron and have wondered if this iron might not have been derived from the phagocytic reticular cell Bessis' excellent electron micrographs show clearly that the reticular cell is func-

tioning as a very real mother cell to the normoblasts which are receiving their iron from it by a process which he compares with nursing.

If the reticular cell of the marrow can eat its progeny and feed them as well, then might not this also occur in other situations? Figure 1-3 shows features which may be compared with those of Bessis' story of reticular cells, normoblasts, and iron. The reticular cell contains a complete series of what appear to be lymphocytes in various stages of digestion, and it is surrounded by a

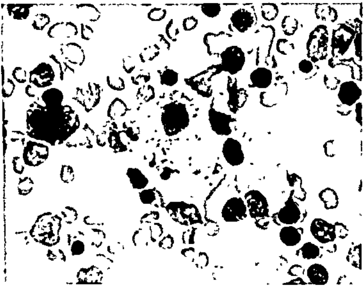


FIG 1 15 Bone marrow from patient with excess storage iron Wright's stain Note reticular cell containing one engulfed red cell and many granules The cells impinging upon the cytoplasmic membrane of the reticular cell include 2 normoblasts, 1 mature and 2 young neutrophils, 2 lymphocytes, and 1 plasma cell The 2 cells at the 2 and 3 o'clock positions are probably endothelial cells, the reticular cell itself being perithelial (× 800)

ring of lymphocytes The union of the peripheral ring of lymphocytes and the reticular cell seems intimate White lines which look almost like scratch marks on the three nuclei pushed into the cytoplasm of the reticular cell at the 9 and 11 o'clock positions appear to be real extensions of the cytoplasm of the reticular cell over the body of the lymphocyte Is this reticular cell eating the cells in this ring or feeding them, or does it too have both functions?

Are the perivascular plasma cells feeding (see Figs 1, 2, and 4, Plate 3, of Reference 1 and Figs 1-15 and 1-16 of this chapter)? Perivascular plasma cells contain iron in hemochromatosis,[31, 37, 39] although proof that they phagocytose erythrocytes in this condition is lacking Here we have assumed the iron may

have been transferred from the cytoplasm of the reticular cell to the plasma cells. Does this same type of transfer occur during the production of gamma globulin and antibodies? (In Figs 1-15 and 1-16 one can see a reticular cell of the marrow chosen purposefully to illustrate the variety of cells which seem to adhere to its peripheral cytoplasmic boundaries.)

The transformation of reticular cells to lymphocytes seems well established Now what of the germinal center cells, the rounded forms which vary in size,

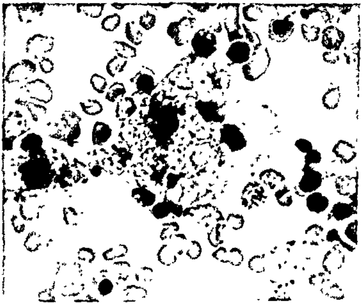


FIG 1-16 Same cells as in Fig 1 15 subsequent to "counterstaining" with Prussian blue The gray cytoplasm of the reticular cell is actually bright blue green (ferritin) The coarse dark granules are even more strongly Prussian blue positive Note iron in neutrophils The normoblasts contain iron, but this is not apparent in the photomicrograph (× 800)

nuclear-cytoplasmic ratio, cytoplasmic basophilia, and nuclear pattern? These appear to be the most actively multiplying cells of the lymph nodes, and these are the cells which cause the most confusion insofar as histopathologic or hematopathologic diagnoses are concerned

STRUCTURE

The various forms of germinal center cells can be seen in Figures 1-1 and 1-2 Germinal center cells are also illustrated by Bessis whose colored plate (Plate XXI, Part I, of Reference 10) shows the varying colors of the inclu-

sions in "reticulum cell macrophages" of a lymph node imprint as well. Heilmeyer and Begemann[41] also show a colored plate of a dry film preparation from a hyperplastic lymph node. In this group of cells, we can name hematopoietic reticular cells, reticular lymphocytes, and immature lymphocytes which are all often classified as lymphoblasts.

In addition to these lymphocytic precursors, plasmablasts and plasma cells are present in remarkably variable numbers. As indicated previously,[1] the immature plasma cells are not always easily distinguished from the more basophilic precursors of lymphocytes Morphologically one can produce convincing series of transitional cells from the large perivascular (perithelial) reticular cell to the plasma cell or from large basophilic hematopoietic reticular cells and/or reticular lymphocytes to plasma cells. The interrelationships of reticular cells, lymphocytes, and plasma cells have been emphasized by Downey. These interrelationships are presented in diagram form by Trowell[20] Two more distinct cell series are included in the schema of Heilmeyer and Begemann (Plate 1/2 of Reference 41).

INTERRELATIONSHIPS

The implications of the interrelationships in regard to the formation of antibodies have recently been discussed by Fagraeus[42] who stated, "The immature plasma cell stage is to me the morphological manifestation of a cell at the point of maximal antibody formation" She stressed "the probability that antibody production commences in young undifferentiated cells, not in plasma cells or mature lymphocytes present in the body before the injection of antigen"

Karyometric analyses of human cells from lymph glands[43,45] are interpreted as indicating that "possibly the only function of basophilic germ centre cells may be the production of cells"[43] and that the basophilic stem cells of the pulp of lymph nodes "belong to the plasma cell series and therefore can be called plasmablast and proplasmablast"[43] The illustrations in these papers are excellent, but it is difficult to see how the problem can be solved on the basis of nuclear volume since the latter can show great variation For example, large and small myelocytes are easily identified, and I have often seen the late telophase of mitosis which would result in two "daughter" normoblasts with apparently remarkably different nuclear volumes The presence of plasmablasts[46,47] in the blood in German measles, infectious hepatitis, and infectious mononucleosis is well recognized However, I was surprised to find plasma cells in the blood in one case of agammaglobulinemia More interesting was a recent case (etiology of condition unknown) with a remarkable peripheral plasmacellular reaction comparable to that of German measles in which the circulating immature plasma cells did not contain gamma globu-

lin.[49] I have come to wonder if the cells common to peripheral plasmacellular reactions and comparable to those that cause the confusion by virtue of their presence in germinal centers may not be different from the plasma cells that are so clearly perivascular in the marrow and other organs

The transition of large hematopoietic reticular cells to reticular lymphocytes to lymphocytes is clearly evident in lymph node imprints from infectious mononucleosis and chronic lymphatic leukemia.[8] In these cases the transitional forms resemble those of hyperplastic nonleukemic nodes, but the leukocytoid lymphocytes of Downey[49] are, of course, more prominent in the nodes from infectious mononucleosis.

In the acute and subacute lymphatic leukemias, the transitional forms appear to progress from narrow-bodied reticular cells to lymphoblasts to immature lymphocytes to lymphocytes[1, 8, 50] (See Figs. 1-13, 1-14.*) The lymphoblast of acute lymphatic leukemia is remarkably similar to the myeloblast of acute myelogenous leukemia, and this type of cell is uncommon in normal human lymph nodes Downey felt the lymphoblast was an abnormal cell type possibly peculiar to leukemia, representing a cell form resulting, in part at least, from numerous mitoses and lack of subsequent differentiation. The immature lymphocyte, in contrast, is commonly present in normal lymph nodes, and its presence in the marrow[1] has caused me to wonder if it may not represent a transitional form from lymphocyte to myeloblast, thus providing a source of myeloblasts. Yoffey's enthusiasm for this cell type[26, 51, 52] has been most stimulating, and we have compared our marrow specimens as well as our opinions.

With regard to the immature lymphocytes and lymphoblasts, several other interesting observations seem pertinent here Immature lymphocytes seem much more numerous in imprints of the human thymus than in imprints of human lymph nodes[53] Cells resembling the lymphoblasts of acute leukemia are present[53] Immature granulocytes similar in morphology to those of the marrow are present[53, 54] In addition, reticular phagocytes showing features comparable to those shown in Figure 1-3 and occasional large rounded reticular cells are present[54] Plasma cells are also found[53] The presence of colorless to pale-pink granules in the perinuclear cytoplasm of the immature lymphocytes and lymphoblasts of the thymus makes them appear remarkably similar to the immature lymphocytes and lymphoblasts of leukemia[53]

Another interesting observation regarding immature lymphocytes involves two cases of typical chronic myelogenous leukemia (of long duration) which in acute exacerbation gradually lost the steadily increasing number of myeloblasts to show almost complete replacement of the marrow and of the cells of the circulating blood by cells which could be classified as lymphoblasts and

* I am grateful to Dr Norman Nelson of Detroit for the opportunity of having been consulted in respect to this interesting case

immature lymphocytes Only the development of occasional small granulocytes, chiefly basophils, from the cells which resembled immature lymphocytes could be used as confirmatory evidence of previous myelogenous leukemia. *Unfortunately, Sudan black staining of these remarkable cells was not possible at the time of consultation.*

The latter pieces of information suggest that the cell which appears to be a transitional form (immature lymphocyte) between the lymphoblast and the lymphocyte could be remarkably important in normal or abnormal hematopoiesis in general.

Data regarding the cells containing tritium labeled thymidine (H^3-thymidine) which has been incorporated into the DNA of rapidly proliferating cells are now accumulating Currently, it has been shown that circulating nonsegmented cells of some type are labeled and are thus cells which would be expected to divide.[6] It is not possible to be absolutely certain of the cell types from the figures or the text, but I should expect them to be immature lymphocytes (their description and Fig. 1-e of Reference 6) and reticular lymphocytes (Type III, Downey). The Group 5 cells of Bond and associates[6] which appeared to be damaged could be basket cells (not identifiable), damaged immature lymphocytes, or even cells comparable to the cells from the blood of leukemic reticuloendotheliosis illustrated in this chapter (Figs. 1-9 through 1-12). It should be re-emphasized that the reticular cell that seems almost to lack cytoplasm can be found along with the histiocyte in the blood septicemia and subacute bacterial endocarditis, and I have seen it in the blood in infectious mononucleosis which presented with an initial histiocytic[1] picture. I have not seen mitoses comparable to those shown in Figures 1-11 and 1-12 in nonleukemic blood. I have frequently seen mitoses in reticular lymphocytes in the blood in infectious mononucleosis, and I have occasionally seen mitoses in plasmablasts in acquired hemolytic anemia. Mitoses in reticular lymphocytes and plasmablasts do occur in the blood in the absence of leukemia I should not be surprised to find a mitosis in an immature lymphocyte in some of the lymphocytoses of infancy but do not recall having seen one That the real unidentifiable basket cells may be premitotic cells is a surprise I should tentatively conclude that the "mobile pool of primitive progenitor cells [6] consists of cells comparable to those found in the germinal centers of lymph nodes. It is difficult to conceive of the reticular lymphocytes, plasmablasts, and immature plasma cells as truly multipotential cells under most circumstances, but the number of these cells found by Bond and associates[6] suggest that very probably the label was bound to these cell types that surely are capable of division The reticular cell that seems to lack cytoplasm is totally acceptable as a multipotential stem cell. The immature lymphocyte may be similarly multipotential and may function as a transitional form between the lymphocyte and other blast forms

SUMMARY

The hypothesis of the origin of lymphocytes from a previously phagocytic reticular cell that contains degradation products of phagocytosed lymphocytes and thus would serve to explain the conservation of labeled DNA and the curiously "long" life span of lymphocytes has been examined and supported by new illustrations from dry film material.

The intimate union of cytoplasm of the lymphocytes surrounding the phagocytic reticular cells has been compared with a similar union between normoblasts and reticular cells. The latter allows normoblasts to receive ferritin from the reticular cells by a process comparable to nursing (Bessis).[30a] I suggest that although the reticular cells do eat the lymphocytes, the lymphocytes probably also get sustenance from the reticular cells. That other perireticular cells (plasma cells) may also receive materials essential to their growth or function in this way is equally obvious, and the importance of the perireticular position of plasma cells to their possible function in antibody production deserves further investigation.

The developmental potentialities of lymphocytes have been mentioned.

The confusion regarding cell types in the germinal centers has been discussed The fact that the morphologically immature cells are occasionally present in normal blood and are numerous in the blood in lymphocytic reactions has been emphasized by labeling techniques These cells, known to be capable of mitosis, show incorporation of tritiated thymidine into DNA and can be studied by radioautography The fact that immature lymphocytes (narrow-bodied cells morphologically intermediate between lymphocyes and small blast forms comparable to the lymphoblasts of acute lymphatic leukemia) occur in the lymph nodes, bone marrow, thymus, and, in small numbers, in the blood has been re-emphasized in order that their role as tritium-labeled cells might be clarified.

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(not thoroughly investigated in some of the lower forms), and three types of granulocytes. However, *purely lymphocyte-forming organs* (lymphatic nodes and nodules) are found only in the mammals and in some water birds in which they are limited to small, paired organs in the cervical and lumbar regions. In adult birds *lymphatic tissue* appears as the white pulp of the spleen and as nodules in parts of the connective tissue, in the lamina propria of the gastrointestinal tract, in the bone marrow, and often in the liver.

Below the mammals and birds there is no exclusively lymphatic tissue, that is, there is no separation of lymphatic and myeloid tissues. The lymphocytes develop intermixed with developing granulocytes in a variety of situations, hence the name *lymphomyeloid tissue*. A striking example of this is in the subcapsular tissue of the liver of urodeles; the same process occurs in the liver of human embryos.

In general, in the lower vertebrates, erythropoiesis occurs in organs other than those in which the lymphomyeloid tissue is found. In these forms, just as in reptiles and birds, erythropoiesis is usually intravascular.

It is noteworthy that, within a given vertebrate species, the large and small stem cells are morphologically indistinguishable, although there may be some characteristic cytologic differences between species.

Just as the development of purely lymphocyte-forming tissues and organs occurs late in phylogeny, so, too, does it appear late in mammalian embryonic development. Nevertheless, it must be noted that (1) lymphocytes are present in the blood of the lower vertebrates and (2) lymphocytes, even small ones, develop very early in mammalian embryos.

EMBRYOGENESIS OF MAMMALIAN BLOOD CELLS

In the mammalian embryo (including that of man) blood cells develop in and from the mesenchyme in a succession of sites, appearing first in the yolk sac and the head and later in other parts of the body Hematopoiesis next appears in the liver, then in the bone marrow, and finally in lymph nodes and spleen In some species the thymus may be very important, as Dr. Matthew Block tells me for his developing opossums.

In all these situations, the hematopoietic process is much the same except that the primitive erythrocytes are formed only in the body mesenchyme and yolk sac (Slide 1). With this exception, all the various types of blood cells form in each of the situations, but the proportions of the different cell types vary from site to site There may be minor variations between species Thus, definitive erythrocytes are formed in great numbers in the yolk sac of the cat, guinea pig, and rabbit but only in small numbers in that of man and the rat (Slides 2-4) In all mammals definitive erythrocytes are formed extensively in the liver (Slide 5) and bone marrow (Slides 6 and 7). In most mammals megakaryocytes and granulocytes are common in yolk sac and liver (Slide 5)

CHAPTER 2

THE EMBRYOGENESIS OF LYMPHOCYTIC TISSUE

WILLIAM BLOOM

DEFINITION OF TERMS

It is impossible to define to the satisfaction of all the terms "lymphocytes" and "lymphocytic tissue" It would be relatively simple if we agreed that the term "lymphocyte" meant the smaller lymphocytes as represented by the non-monocytic, nongranular leucocytes which gain access to the peripheral blood. This definition is, however, insufficient since it does not consider the relation of these "lymphocytes" to the larger free stem cells in the various hemato-poietic tissues—cells which rarely, if ever, enter the blood stream under normal conditions There is less difficulty in defining *lymphocytic tissue* if by this is meant the cellular stroma and the included lymphocytes and free phagocytes which comprise the lymph nodes, white pulp of the spleen, and the solitary and aggregated nodules (and tonsils) of the lamina propria of *gastrointestinal, respiratory, and, occasionally, generative tracts of postnatal* mammals But we face further problems in attempting to delimit the thymus, the splenic red pulp, and the accumulations of lymphatic tissue in normal and abnormal bone marrow

Nevertheless, in any discussion of the embryogenesis of lymphocytes and lymphocytic tissue, one cannot avoid the obligation of at least trying to define these terms To this end, it is necessary to consider briefly the hematopoietic tissues of the vertebrates in which there are some striking and illuminating parallels in the phylogenetic and ontogenetic development of blood cells (see Jordan[5])

COMPARATIVE HEMATOPOIESIS IN VERTEBRATES

The blood of practically all vertebrates contains erythrocytes, nucleated thrombocytes (nonnucleated platelets in mammals), lymphocytes, monocytes

in which case they or their progeny are called lymphocytes, or they will develop into erythroblasts, some myelocytes and a few megakaryocytes. Later the typical division of the organ into red and white pulp develops. In man the formation of the myeloid elements ceases under normal conditions but appears again in extramedullary myelopoiesis

I have not discussed explicitly the nature of the primitive free stem cells of the several hemocyte-forming areas and their relation to the question of what is a lymphocyte. I know of no morphologic criteria by which these free mesenchymal cells can be separated into various cell lineages until some of them develop specific granules and become granulocytes, others elaborate hemoglobin and become erythrocytes, and until still others, for example, become plasma cells as shown by the specific fluorescent antibody technique I, for one, find it impossible to discriminate several types of free stem cells when I study myelocytes developing along with lymphocytes (1) in a lymph node primordium, (2) in the more obvious lymphomyeloid tissue of the other embryonic mammalian organs as yolk sac, liver, or bone marrow, (3) in lymphomyeloid tissues of the lower vertebrates, (4) in the bone marrow with prominent lymphatic nodules in birds, or (5) in extramedullary myelopoiesis in postnatal mammals when there is a reversion to the mixed lymphomyeloid tissue.

It seems to me that the sum of the comparative, embryologic, and experimental cytologic evidence clearly points to the conclusion that in all blood cell forming situations the mesenchyme turns into free stem cells which give rise to all the types of blood cells in all the primordia and that with the progressive development of the embryo there is an apparent gradual development, in some mammals, of a functional separation in postnatal life of myeloid and lymphatic tissues. But this separation is not always permanent, for in certain diseases in man and as a result of certain experiments, both of these tissues may revert to the mixed lymphomyeloid or lymphomyeloerythropoietic tissue of the embryo

DESCRIPTIONS OF ILLUSTRATIONS

Fourteen colored lantern plates illustrated some of the stages of the embryogenesis of mammalian blood cells in the original presentation of this chapter Seven of the slides were copied from published colored plates in articles by A A Maximow,[8, 9] W Bloom,[1, 2] and W Bloom and G W Bartelmez[3] The other seven slides were color photomicrographs made expressly for the presentation It has not been possible to reproduce them in color here It is better to refer specifically to the original publications of the drawings rather than to reproduce them here in black and white The color photomicrographs would be useless in black and white because the myelocytes developing in dense masses of lymphoid cells would not show

and, of course, in the bone marrow. As we shall see, myelocytes are common in the developing lymph nodes of human embryos, erythroblasts also occur, but megakaryocytes are rare

In all these hematopoietic foci, the process begins with the contraction of outstretched mesenchymal cells into spherical, ameboid cells of various sizes characterized by relatively little cytoplasm, large nucleoli, and—in fixed and stained preparations—an intense cytoplasmic basophilia, more marked in the larger than in the smaller forms. These free mesenchymal cells (stem cells or "blasts") give rise to all types of blood cells in all of the successive sites of blood cell formation. Occasionally an outstretched mesenchymal cell seems to round up and turn directly into a granulocyte without passing through a free, basophil, stem cell stage

EMBRYOGENESIS OF HUMAN LYMPHOCYTIC TISSUE

In human embryos of 30 mm, the first lymph nodes begin to develop in the walls of the cervical lymph sacs, later in the other lymph sacs, and still later along peripheral lymphatics. In all the sacs, the process is much the same cords of mesenchyme bulge into the lumen of the sacs and give rise to some free macrophages and foci of intensely proliferating stem cells. These are of all sizes, although the smaller forms predominate. Because of their location these free stem cells must be called lymphocytes (Slide 8) From the stem cells (i e, lymphocytes) there develop eosinophil and neutrophil myelocytes, although in smaller numbers than in the embryonic bone marrow (Slides 9-12) Sometimes these small myelocytes show mitoses These myelocytes have nuclei indistinguishable from those of the surrounding lymphocytes. Occasional megakaryocytes and small groups of erythroblasts also occur

It is rare to find the specific leukocyte granules in the larger lymphoid cells Very occasionally, eosinophil granules are found in cells with exceedingly pale cytoplasm and with large nuclei lacking the large nucleoli characteristic of stem cells They are probably mesenchymal cells giving rise directly to myelocytes

As the embryo grows, the lymphatic tissue, except in some of the laboratory mammals, normally ceases to produce myeloid elements, although in ectopic myelopoiesis this process may become active again (Slide 13)

In the embryonic thymus eosinophil myelocytes are found in small numbers, their nuclei are identical with those of the surrounding smaller lymphocytes (Slide 14) A few developing erythroblasts and megakaryocytes have also been reported

The primordium of the spleen begins primarily as a vascular meshwork of mesenchyme which gives rise to free stem cells These may persist as such,

6. MAXIMOW, A. A. Untersuchungen über Blut und Bindegewebe. I. Die frühesten Entwicklungsstadien der Blut—und Bindegewebszellen beim Säugetierembryo, bis zum Anfang der Blutbildung in der Leber. *Arch. f. mikr. Anat.* 73:444, 1909.
7. MAXIMOW, A. A. Untersuchungen über Blut und Bindegewebe. II. Ueber die Histogenese der Thymus bei Säugetiere. *Ibid.* 74:525, 1909.
8. [illegible]
9. [illegible] *der mikroskopischen Anatomie des Menschen*, ed. by v. MOELLENDORFF. Berlin, J. Springer, 1927, vol. 2, part 1, p. 232.

SLIDE 1: Stages in the transformation of mesenchymal cells into the first free stem cells and the development of the latter into primitive erythrocytes in the yolk sac and body mesenchyme of several species of mammalian embryos (A. A Maximow, Plate XVIII.[6] Reproduced in Bloom.[2])

SLIDES 2 and 3: Various stages in the development of the free stem cells, in the human yolk sac, into primitive erythrocytes, small and medium-sized lymphocytes, phagocytes, megakaryocytes, and eosinophilic and neutrophilic myelocytes Figure B shows development of primitive and definitive erythroblasts in adjacent foci in a slightly older human yolk sac. (W. Bloom and G. W. Bartelmez, Plates 1 and 2.[3])

SLIDE 4: Colored photomicrograph of several large stem cells and one medium-sized one in yolk sac of human embryo.

SLIDE 5: Colored photomicrograph of hematopoiesis in human embryo (70 mm.) liver near the capsule. Eosinophilic and neutrophilic myelocytes are intermixed with medium-sized lymphocytes, one of which is in mitosis Nearby are one very large stem cell and a group of polychromatic definitive erythroblasts.

SLIDE 6 Development of large, medium-sized, and small stem cells in the mesenchyme of primitive bone marrow in embryos of several laboratory mammals (A A. Maximow, Plate II [8])

SLIDE 7 Development of stem cells of various sizes, definitive erythroblasts, and myelocytes in primitive human embryonic marrow (A. A Maximow, Figs 105 and 106 [9])

SLIDE 8 Two stages in early human lymph node primordia showing development of large and many small stem cells (lymphocytes) from mesenchyme (A A Maximow, Figs. 107 and 108 [9])

SLIDES 9-12 Colored photomicrographs of several stages in developing lymph nodes of a human embryo of 70 mm These formed an important part of the presentation as they showed eosinophilic and neutrophilic myelocytes developing in moderate numbers in the lymphatic tissue These granulocytes have nuclei indistinguishable from those of the surrounding smaller lymphocytes

SLIDE 13 Development of eosinophilic and heterophilic myelocytes from small lymphocytes within germinal centers of white pulp of spleen and a nodule of lymph node from guinea pigs (W Bloom, Plate I [1])

SLIDE 14 Colored photomicrograph of three eosinophil myelocytes in human embryonic thymus

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1 BLOOM, W The hemopoietic potency of the small lymphocyte *Folia haemat* *33* 122. 1926

2 BLOOM, W "Embryogenesis of Mammalian Blood," in *Handbook of Hematology*, ed by DOWNEY, H , New York, Paul B Hoeber, Inc , 1938, vol. 2, p 865

3 BLOOM, W , and BARTELMEZ, G W Hematopoiesis in young human embryos *Am J Anat* *67* 21, 1940

4 DOWNEY, H The structure and origin of mammalian lymph nodes and their [illegible], 1922

5 [illegible] *Hematology*, ed by [illegible], p 699

by using the supravital (neutral red and Janus green) staining method in conjunction with the phase contrast microscope.[5, 6]

Numerous investigators have studied the lymphocytes of the blood[5, 6, 14, 24, 31, 32, 35, 48, 71] and lymphatic tissues[9, 47, 51, 94] with the phase microscope. The morphologic characteristics of the lymphocyte and its progenitors as revealed by the phase microscope will be briefly summarized and illustrated in both the normal and leukemic conditions.

Normal Lymphocytes

The most primitive lymphoid cell observed in lymphatic tissue is the lymphoblast (Figs. 3-8, 3-9), a rather large, rounded cell (18–21 μ) with a large, round or slightly indented, moderately vesicular nucleus. It possesses a thin nuclear membrane and two to four ovoid or irregular-shaped nucleoli The cytoplasm is deeply basophilic and appears yellowish or opaque with the phase microscope. A moderate number of small rodlike or spherical mitochondria are scattered throughout the cytoplasm and tend to localize near the nuclear hof and distal portion of the cytoplasm. A rosette of granules or vacuoles surround the cytocentrum and stain orange-red with neutral red.

As the lymphoblast undergoes further differentiation, there is a condensation of the chromatin and a thickening of the nuclear membrane. Initially, there is little or no change in the size of the cell or in the degree of cytoplasmic basophilia or number of organoids. A cell exhibiting these characteristics is termed a large lymphocyte (Figs. 3-6, 3-7) and can be distinguished from the lymphoblast primarily by small differences in the chromatin pattern of the nucleus.

As the lymphocyte continues to mature (Figs 3-4, 3-6), it gradually decreases in size, degree of cytoplasmic basophilia, number of mitochondria and *cytoplasmic granules, and number and size of nucleoli and exhibits a* further condensation or clumping of the chromatin of the nucleus.

The small mature lymphocyte (Figs 3-1, 3-2, 3-3, 3-5) has a round or ovoid contour (10–12 μ) with well-defined borders, a small amount of cytoplasm surrounding a slightly indented or oval nucleus. The nucleus has a dense, coarse chromatin pattern with a prominent nuclear membrane and usually contains a small nucleolus which tends to be obscured by the dense "nucleolar associated chromatin." A small highly refractile nonstaining lipoid droplet[53] (the body of Gall) is seen occasionally in the cytoplasm near the nuclear hof Small nonrefractile elongate or spherical mitochondria are scattered throughout the cytoplasm concentrating near the nuclear hof. The perinuclear zone and cytocentrum is less basophilic and opaque than the remaining homogeneous hyaloplasm The lymphocyte exhibits a charac-

CHAPTER 3

CYTOCHEMISTRY OF THE LYMPHOCYTES

Phase Microscope Studies

G. ADOLPH ACKERMAN

It is apparent that we are entering a new phase of normal and pathologic cellular morphology resulting from the rapid advances in the fields of microscopy and histochemistry. It has become necessary for the pathologist, hematologist, and hematologic investigator to be familiar with the wide variety of new methods now available to him for aid in diagnosis of disease, studying the process of cellular differentiation, determining the morphologic and chemical alterations in cells in various diseases, and in determining the effects of various drugs and therapeutic agents upon their structure, chemistry, and metabolism

PHASE MICROSCOPY

The recent advent of the phase microscope has renewed interest in the morphologic structure of the lymphocyte since it has become possible to examine lymphocytes in the blood and lymphatic tissues in the living condition unaltered by drying, fixation, or staining It has proved possible to recognize in the living cells studied in this manner all the structures and structural detail described with the Romanovski stains and, in addition, to visualize other cellular entities not observed by the Romanovski methods, e g , cytoplasmic organelles and inclusions Cytoplasmic movements and cell motility can be observed with the phase microscope, as can intracellular alterations during the process of cell degeneration and death Experience has indicated that the resolution of the organelles present in the lymphocyte as well as in other cells of the blood and hemopoietic system can be increased

teristic type of slow ameboid movement in which the nucleus advances ahead of the cytoplasm, the so-called hand-mirror movement.

Estimating the maturity of the lymphocyte from cell size alone is not sufficient and may lead to erroneous interpretation of the lymphocytes' age. Experience has indicated that the most reliable criteria for determining the relative age of the lymphocyte are obtained by the correlation of several morphologic features, e.g., degree of cytoplasmic basophilia, number of mitochondria, cell size, and the chromatin pattern of the nucleus.[5, 112]

Leukemic Lymphocytes

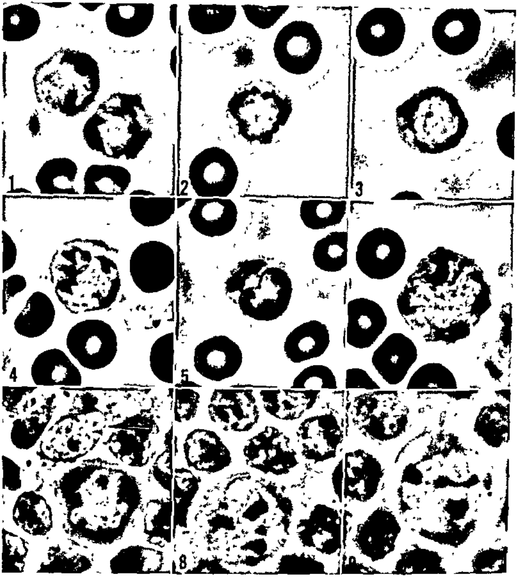
Lymphocytic Leukemia. Lymphocytes in chronic lymphocytic leukemia (Figs. 3-10, 3-11) are small (9–12 μ) and possess a more dense chromatin pattern than is usually seen in normal small lymphocytes. The hyperchromatic appearance of the nucleus is dependent, in part, upon the increase in the number of small irregular nucleoli and the "nucleolar associated chromatin." The contour of the nucleus may vary from a smooth to an irregular rounded form. The cytoplasm of the small lymphoid cells in chronic lymphocytic leukemia tends to be scantier than in the normal lymphocytes. The number of mitochondria and cytoplasmic granules as well as the degree of basophilia of the cytoplasm may frequently be slightly greater in the leukemic than in the normal small mature lymphocyte.

A complete maturation series of lymphoid cells can occasionally be seen in the blood of certain patients with lymphocytic leukemia (subacute variety). These immature lymphoid cells tend to exhibit more dense nuclei and have a somewhat greater number of mitochondria and nucleoli than the small lymphocytes of lymphocytic leukemia and comparable normal lymphoid cells at similar stages of maturation. In these cases larger lymphoid cells exhibiting morphologic features of blast cells (Fig. 3-11) are present in relatively small numbers in contrast with the small lymphocytes which predominate. The morphologic features of the lymphoblasts seen in lymphocytic leukemia differ from cells identified as lymphoblasts in normal lymphatic tissue.

FIG 3-7 Normal human lymph node Note the large lymphocyte with 2 nucleoli, basophilic cytoplasm, and spherical mitochondria localized in the nuclear hof and distal portion of the cytoplasm. Several small lymphocytes are present as well as a stellate reticulum cell *(arrow)* Supravital preparation of lymph node scrapping

FIG 3 8 Normal human lymph node Lymphoblast with prominent nucleoli Mitochondria present in the nuclear hof and swollen mitochondria in the distal portion of the cytoplasm Supravital preparation of lymph node scrapping

FIG 3 9 Normal human lymph node Lymphoblast containing few mitochondria and cytoplasmic granules Cytoplasm appears opaque and deeply basophilic Supravital preparation of lymph node scrapping



Figs 3-1 through 3-9 Photographed from supravital films with the phase contrast microscope (dark medium contrast) (× 2000, reduced 25 per cent)

Fig 3-1 Two small lymphocytes Note the filamentous mitochondria and spherical neutral red staining granules

Fig 3 2 An "old" lymphocyte containing spherical mitochondria and a few neutral red granules Cytoplasm is less basophilic and opaque than the lymphocytes in Fig 3 1

Fig 3 3 Small mature lymphocyte Note small irregular nucleolus

Fig 3-4. Medium-size lymphocyte with localization of mitochondria and granules *around the clear cytocentrum*

Fig 3-5 Small young lymphocyte with indented nucleus The cytocentrum is rather clear, and small neutral red granules localize in this region Mitochondria are peripheral to the cytocentrum

Fig 3 6 Large young lymphocyte containing a moderate number of granules and both spherical and filamentous mitochondria

Lymphoblastic Leukemia. True lymphoblastic leukemia is an extremely rare entity and differs from lymphosarcoma and leukolymphosarcoma with which it has been frequently confused. The cells observed in lymphoblastic leukemia (Figs. 3-17, 3-18) are rather large (11–18 μ), round or oval cells with large, round, moderately vesicular nuclei, a prominent nuclear membrane, and one to three large prominent nucleoli. Mitochondria are numerous and appear considerably larger than those seen in lymphocytic leukemia or lymphosarcoma. The mitochondria localize near the nuclear hof and in the more distal part of the cytoplasm. Neutral red staining granules or vacuoles localize near the cytocentrum and stain an orange-red color. These cells exhibit slight hand mirror type motility characteristic of lymphoid cells.

Lymphosarcoma. The pathologic lymphoid cells characterizing lymphosarcoma in lymphatic tissue and in the blood (so called leukolymphosarcoma) are morphologically identical; however, these cells may exhibit considerable variation in morphology (Figs. 3-12 through 3-16). The classic lymphoid cells present in lymphosarcoma appear rather small but actually are slightly larger (12–14 μ) than the normal small lymphocytes and those of lymphocytic leukemia. The lymphosarcoma cells possess a vesicular nucleus with a thin nuclear membrane, several prominent nucleoli, and a small rim of opaque, moderately basophilic cytoplasm. Not infrequently the nuclei of the lymphosarcoma cells appear hyperchromatic (Fig 3-12), become markedly indented, and may assume a lobed or clover-leaf nuclear form. It is possible that these changes could be correlated with the increased content of DNA which has been observed in the cells of lymphosarcoma.[80] The small spherical mitochondria tend to localize near the nuclear hof, and a small number of granules which stain orange-red with neutral red occur near the region of the cytocentrum Highly refractile, nonstaining lipoid droplets may occur in variable numbers in the cytoplasm of the lymphosarcoma cells in certain patients with this disease. The lymphoid cells of lymphosarcoma have a relatively high mitotic index in contrast to those of lymphocytic and lymphoblastic leukemia A comparison of the morphologic structure of the cells of lymphosarcoma reveals that they exhibit relatively few characteristics in common with lymphoblasts seen in normal lymphatic tissue

Occasionally in certain patients with lymphosarcoma, a number of cells exhibit considerable variations in size with slight variations in the relative amount of cytoplasm, cytoplasmic basophilia, and in the number of mitochondria These observations suggest a maturation series in the cells of lymphosarcoma since small, medium, and large forms (Fig 3-15) can be distinguished.



Fig 3 10 through 3 18 Photographed from supravital films with the phase contrast microscope (dark medium contrast) (× 2000, reduced 25 per cent)

Figs 3 10 and 3 11 Lymphocytes from chronic lymphatic leukemia Note hyperchromatic nuclei, small nucleoli, and "nucleolar associated chromatin" Cytoplasm is scanty and contains numerous small spherical mitochrondria and a few slightly larger neutral red staining granules A lymphoblast is present in Fig 3 11, possessing a prominent cytocentrum surrounded by granules, and mitochondria are scattered throughout the cytoplasm

Figs 3-12 through 3-16 Variations in the morphologic structure of the lymphocytes of leukolymphosarcoma Granules and spherical mitochondria localize in the hof Cytoplasm is basophilic and opaque The chromatin pattern is variable, appearing either vesicular (Figs 3 13, 3-15, and 3-16) or hyperchromatic (Figs 3 12 and 3-14) A large immature lymphosarcoma cell is illustrated in Fig 3-15

Figs 3-17 and 3 18 Lymphoblasts from acute lymphoblastic leukemia Note vesicular nuclei, large prominent nucleoli, thin nuclear membrane, and a large number of cytoplasmic organelles, primarily mitochondria

The Feulgen nucleal reaction has been combined with microspectrophotometry in order to determine quantitatively the amount of DNA in normal and leukemic lymphocytes.[63, 79, 80] These data[63, 80] indicate that the normal lymphocytes of the blood and the majority of those of lymphatic tissue contain the diploid amount (2n) of chromatin as do other somatic cells. Some of the lymphoid cells in lymphatic tissue possess intermediate values of chromatin between the diploid and tetraploid (4n) amount suggesting a premitotic build up of DNA in the immature lymphocytes.[80] In six patients with chronic lymphocytic leukemia, the lymphocytes were found to contain the normal (2n) amount of chromatin, while those from three patients with chronic lymphocytic leukemia and four of five patients with acute lymphocytic leukemia were found to contain between the normal diploid and the tetraploid amount of chromatin[80] These observations suggest that most of the lymphocytes in chronic lymphocytic leukemia are intermitotic, while those of acute lymphocytic leukemia are either in the process of mitosis with premitotic synthesis of DNA or actually may exhibit polyteny[80] Confirmation of these observations is awaited with great interest.

Pentose Nucleic Acid (PNA or RNA). The basophilic staining property of the cytoplasm and nucleoli of various blood cells following staining with certain basic dyes has been recognized for many years[30, 49, 59, 66, 110] Both microspectrophotometry and selective abolition of basophilia with the enzyme ribonuclease[22] have indicated that this basophilic substance is a nucleoprotein, namely RNA Histochemical studies on normal lymphocytes (Figs 3-19, 3-20) and on those from various hematologic dyscrasias have shown that cytoplasmic basophilia[112] or more specifically RNA varies inversely with the age of the lymphocyte. This substance is formed in large amounts in the lymphoblasts and gradually decreases during the maturation of the lymphocyte.[1, 112] It seems probable that the RNA plays an important role in the formation of cellular proteins and globulins[60] After injection of antigen into an animal, the RNA content of the immature cells increases first in the altered reticular cells then in the young lymphocytes while the DNA content remains constant[36, 49]

Methyl green-pyronin has been widely employed in histochemistry for the simultaneous visualization of DNA and RNA[78] Methyl green acts as a selective stain for highly polymerized DNA present in the chromatin of the cell, while pyronin has an affinity for RNA of the cytoplasm and nucleoli. The RNA nature of the cellular substances is confirmed by selective extraction with ribonuclease Results obtained by the methyl green-pyronin method on the lymphocytes[36, 49, 78] in the blood and lymphatic tissue are in agreement with those described earlier

Differences in methyl green stainability between granulocytes and lympho-

HISTOCHEMISTRY

Recent advances in the field of histochemistry have added considerably to our knowledge of the chemical structure of the lymphocyte and have given some insight into the chemical alterations in these cells in disease. Histochemistry not only provides a method for chemically identifying and classifying various normal and abnormal cells but provides one of the most important approaches for determining the underlying causes of disease and cellular differentiation. Few detailed studies have been made concerning the histochemistry of the lymphocyte either in the normal or diseased conditions. Although lymphatic tissues have been subjected to a large number of histochemical procedures, all too frequently observations are histologic rather than cytologic in nature. Not infrequently histochemical reactions are described as being either present or absent in a given cell or tissue, and the reactive substance is not localized to the morphologic structures within these cells. Considerable data have been derived from the study of leukemic cells without attempting to correlate these findings with comparable cells in the normal condition. These facts coupled with the development and improvement in histochemical procedures and inherent difficulties in various methods have caused considerable variations in the literature concerning the histochemical characterization of the lymphocyte. An attempt has been made to summarize briefly the most important observations concerning the histochemistry of the lymphocyte in health and disease.

Nucleoproteins

Deoxypentosenucleic Acid (DNA). Both the Feulgen nucleal reaction[1, 2, 41, 56] and ultraviolet microspectrophotometry[65, 79, 80] have established the presence of DNA in the chromatin of the nuclei of the lymphocytes as well as in the nuclei of other cells in the plant and animal kingdom. Morphologically, the nuclei of the small lymphocytes stain rather intensely and exhibit a dense, coarse, Feulgen-positive chromatin pattern, while the more immature lymphocytes and lymphoblasts possess a more delicate chromatin pattern and stain less intensely than the small mature lymphocytes.[1] Frequently, the Feulgen-positive "nucleolar associated chromatin" is very apparent surrounding the Feulgen-negative nucleoli of the lymphoid cells. The nuclei of the lymphocytes in chronic lymphocytic leukemia have a coarse, darkly stained chromatin in contrast to the lymphocytes in lymphosarcoma which usually stain lighter and have a more uniform distribution of chromatin.[1] The enzyme desoxyribonuclease will selectively remove the stainability of the lymphocyte with basic dyes[59, 94] or with the nucleal reaction.[9]

The Feulgen nucleal reaction has been combined with microspectrophotometry in order to determine quantitatively the amount of DNA in normal and leukemic lymphocytes[63, 79, 80] These data[63, 80] indicate that the normal lymphocytes of the blood and the majority of those of lymphatic tissue contain the diploid amount (2n) of chromatin as do other somatic cells Some of the lymphoid cells in lymphatic tissue possess intermediate values of chromatin between the diploid and tetraploid (4n) amount suggesting a premitotic build up of DNA in the immature lymphocytes.[80] In six patients with chronic lymphocytic leukemia, the lymphocytes were found to contain the normal (2n) amount of chromatin, while those from three patients with chronic lymphocytic leukemia and four of five patients with acute lymphocytic leukemia were found to contain between the normal diploid and the tetraploid amount of chromatin.[80] These observations suggest that most of the lymphocytes in chronic lymphocytic leukemia are intermitotic, while those of acute lymphocytic leukemia are either in the process of mitosis with premitotic synthesis of DNA or actually may exhibit polyteny.[80] Confirmation of these observations is awaited with great interest.

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Differences in methyl green stainability between granulocytes and lympho-



FIGS 3 19 through 3 27 Histochemistry of normal lymphocytes (× 2000, reduced 25 per cent)

FIG 3 19 Normal lymphocyte exhibiting basophilic cytoplasm Methyl alcohol fixation Wright's stain

FIG 3 20 Normal lymphocyte exposed to ribonuclease for 10 minutes followed by Wright's stain Cytoplasmic basophilia has been removed by enzymatic digestion

FIG 3-21 Normal lymphocyte stained by the periodic acid Schiff method Note the small faintly stained PAS positive granules in the cytoplasm

FIG 3 22 Normal lymphocyte stained for masked lipids Small sudanophilic granules can be distinguished in the cytoplasm Nuclear staining may represent nuclear lipid Sudan black following unmasking with acetic acid

FIGS 3 23 and 3-24 Normal small lymphocytes stained for protein bound sulfhydryl groups by the DDD method of Barrnett and Seligman. Sulfhydryls are localized in the cytoplasm and nucleoplasm while the chromatin is essentially unstained

cytes (chronic lymphocytic leukemia) have been observed following exposure to ribonuclease in that the granulocytes were methyl green-labile and the lymphocytes methyl green-resistant.[58, 103] Further investigations are necessary to substantiate these differences in methyl green staining.

Proteins and Amino Acids

Protein-bound amino groups can be demonstrated in the cytoplasm and nuclei of the lymphocytes (Fig. 3-25) in blood films[9] following Weiss's method[109] for these reactive groups. The cytoplasm stains moderately in contrast to the faint reaction of the nucleus of these cells (Fig 3-25). The cytoplasm is more intensely stained in the larger lymphocytes than in the small lymphocytes, and it appears that this staining reaction tends to parallel the RNA content of the cytoplasm of these cells[9] In most instances the cytoplasm and nuclei of the lymphoid cells of the spleen stain strongly for protein-bound amino groups[109]

Only traces of histidine can be localized in the cytoplasm of the lymphocyte in blood films[9] following the reaction of Burstone. Arginine can be demonstrated in the cytoplasm, nuclei, and nucleoli of the lymphocytes in both blood[108] and lymphatic tissue[9] and appears to parallel the intensity of the reactions for nucleoproteins[9] Tyrosine cannot be demonstrated in the lymphocytes of the blood following the Millon reaction.[106] At the present time protein reactions appear to afford little help in the differential diagnosis of lymphatic diseases.

Sulfhydryl Groups

Lymphocytes of the blood and lymphatic tissue exhibit a relatively weak reaction for sulfhydryl groups following the DDD method of Barrnett and Seligman,[4, 8, 9, 12, 18, 97] the RSN method of Mauri and others,[26, 66] and the older ferri-ferrocyanide procedure[9, 18, 97] Normal lymphocytes in both the blood (Figs. 3-23, 3-24) and lymphatic tissue (Fig 3-37) reveal a pale, diffuse staining of their cytoplasm and a less intense staining of their nuclei following the DDD[4, 8, 9, 97] and RSN[26, 68] methods However, it appears that

Fig 3-25 Small lymphocyte stained for protein bound amino groups by the method of Weiss The nucleus is only faintly stained in contrast to the darker staining of the cytoplasm and nucleoplasm

Fig 3-26 Lymphocyte stained for nonspecific esterase using the indoxyl acetate substrate Indigo blue crystals have a distribution similar to mitochondria but are not localized in these organelles

Fig 3 27 Demonstration of nonspecific dehydrogenase activity with a modified nitro BT procedure in a normal small lymphocyte Dehydrogenase can be localized to the mitochondria, however, it is uncommon for normal lymphocytes to be stained by this method

the reactive material in the nucleus of the lymphocyte is not chromatin but the nuclear fluid or sap.[9, 97] In normal lymphatic tissue lymphoblasts tend to exhibit a greater amount of cytoplasmic protein-bound sulfhydryl groups than the cytoplasm of the more mature small lymphocytes,[9] although other investigators[29, 68] have shown a gradual increase in sulfhydryl groups during the maturation of the lymphocyte with the RSN technique.

Preliminary studies[8, 9] employing the DDD method for protein-bound sulfhydryl groups suggest that the lymphocytes in lymphosarcoma (Figs. 3-32, 3-33) and in lymphocytic (Fig 3-31) and lymphoblastic leukemia (Fig. 3-34) contain more thiol groups in their cytoplasm and nucleus than normal lymphocytes. However, considerable variations in the amount of reactive thiol groups have been noted in lymphosarcoma,[8, 9] with the lymphosarcoma cells from certain patients reacting very strongly in both the cytoplasm and nucleus (Fig. 3-33). When the RSN method[29] is employed, the lymphocytes in lymphocytic leukemia stain lightly, although the prolymphocytes and lymphoblasts stain somewhat less intensely. Further studies concerning the variations of sulfhydryls in the cellular elements in leukemia and other diseases affecting the hemopoietic system should yield significant results

Following x-radiation, there is a marked reduction in active thiol groups in the lymphoid cells of the lymph nodes and spleen of the rat[4] This reduction occurs within two to four hours after radiation and returns to normal about the twelfth hour. These changes in sulfhydryls parallel the changes in the Gomori alkaline phosphatase reaction, suggesting that these substances may play a significant role in lymphocyte metabolism and radiosensitivity[4]

Carbohydrates

Both glycogen (saliva-labile) and mucopolysaccharides (saliva-resistant) granules can be demonstrated in the cytoplasm of the lymphocyte[1, 2, 4, 10, 37, 63, 64, 67, 70, 74, 85, 99, 114] and its progenitors. Although several methods have been employed for the demonstration of these carbohydrates,[42, 63, 64, 86] the periodic acid-Schiff (PAS) method is the most sensitive and most widely employed[42, 63, 64] PAS-positive granules are scattered throughout the cytoplasm of the lymphocyte (Fig 3-21) with greater numbers of these granules localizing in the cytoplasm in the region of the nuclear hof These granules do not appear to be related to morphologic structures seen in the living cell or following staining with the Romanovski dyes.[9] The number of PAS-positive granules tend to be slightly greater in the more immature lymphocytes[42] and the lymphoblasts Most investigators have attributed the PAS-positive granules to glycogen since they are saliva-labile provided alcholic fixative

was employed. Formalin vapor and Formalin fixatives yield better cellular fixation and staining but tend to bind the glycogen to cellular proteins, making the glycogen resistant to both saliva and purified amylase digestion.[9]

In contrast to the normal lymphocytes, the glycogen content of the lymphoid cells of chronic lymphocytic leukemia (Fig. 3-28) and lymphosarcoma (Fig 3-29) possess relatively large amounts of glycogen.[1, 8, 70, 74, 99, 114] The glycogen content of nucleolated lymphocytes in chronic lymphocytic leukemia is greater than in nonnucleolated lymphocytes,[11] although other investigators[70] have found no differences. The lymphoid cells of acute lymphoblastic leukemia (Fig. 3-30) differ from those of chronic lymphocytic leukemia and lymphosarcoma in that the lymphoblasts possess little or no demonstrable glycogen with the PAS reaction.[8, 70] This finding has been correlated with the absence of phosphorylase activity in the lymphoblasts in acute lymphoblastic leukemia[70] while the phosphorylase activity of the cells of lymphosarcoma and chronic lymphocytic leukemia is present, frequently in relatively large amounts It has been observed that while in most instances the glycogen content of the cells of lymphosarcoma is increased, the cells from certain patients with this disease may exhibit very little glycogen in their cytoplasm.[8, 70] It has been stated[11, 70] that in leukemia glycogen tends to appear relatively early in the maturation of the lymphocyte and increases during cellular differentiation

The presence of glycogen in leukemic cells is a significant finding and can be used with *discretion* as an aid in diagnosis of normal and various lymphocytic diseases, although it must be remembered that glycogen may also be present in increased amounts in certain nonleukemic conditions exhibiting lymphocytoses, e g , infectious mononucleosis [1, 52, 70, 99] Also, glycogen is decreased in lymphoblastic leukemia[8, 70] and in certain cases of lymphosarcoma [8, 70]

Lipids

Although most investigators[17, 46, 50, 62, 74, 83, 86, 92, 93, 100, 113] have failed to demonstrate lipids in either normal or abnormal lymphocytes following staining with fat soluble dyes, e g . Sudan black, Sudan orange, and oil red O, sudanophilic granules can be seen in the cytoplasm of both mature and developing lymphocytes and lymphoblasts in human lymph nodes [1, 2] These sudanophilic granules are small and inconspicuous and tend to localize near the nuclear hof They appear to be slightly more numerous and prominent in the lymphoblast than in the mature lymphocyte and probably represent mitochondria and/or cytoplasmic granules or vacuoles.[1] There is no apparent difference in the number of sudanophilic granules present in the



FIGS 3 28 through 3 36 Histochemistry of leukemic lymphocytes (× 2000, reduced 25 per cent)

FIG 3 28 Lymphocyte from chronic lymphocytic leukemia stained by the periodic acid-Schiff method Note large number of PAS positive cytoplasmic granules

FIG 3-29 Lymphosarcoma cells stained with the periodic acid Schiff method revealing a large number of PAS-positive granules in their cytoplasm

FIG 3 30 Lymphoblast (*arrow*) from acute lymphoblastic leukemia stained by the periodic acid Schiff method PAS-positive granules are very small and are generally less numerous than in normal lymphocytes

FIG 3-31 Two lymphocytes from chronic lymphocytic leukemia stained for protein-bound sulfhydryls by the DDD method Sulfhydryls localize to the cytoplasm and nu clear sap.

FIG. 3-32 Two lymphosarcoma cells stained by the DDD method for protein bound sulfhydryls Sulfhydryls can be localized to the cytoplasm and nucleoplasm

FIG 3 33 Considerable variations in sulfhydryl content of the lymphosarcoma cells

lymphoid cells in lymph nodes from patients with chronic lymphatic leukemia and lymphosarcoma and those in normal lymph nodes[1] In blood films sudanophilic granules have been demonstrated in the lymphoid cells of chronic lymphocytic leukemia,[9, 50] lymphosarcoma,[9] and acute lymphoblastic leukemia.[17]

Masked or bound lipids have been demonstrated in both the cytoplasm and nucleus of the lymphocytes (Fig. 3-22) in fixed blood films after exposure to organic acid and subsequent staining with Sudan black.[3] Following this procedure,[3] numerous, fine, blue-black granules can be readily seen in the cytoplasm of the lymphocyte, and the nucleus becomes sudanophilic but stains a brown color (Fig. 3-22). It is questionable, however, whether this nuclear sudanophilia is due to nuclear bound lipid Following the Baker acid hematin method for phospholipids, the only lymphoid cells observed to contain phospholipids are the lymphoblasts in which they are limited to the mitochondria.[17]

Plasmalogen can be demonstrated in very small amounts in the lymphoid cells of the blood[9, 13, 71] and lymphatic tissues[2, 13] following the plasmal reaction. A small number of tiny, pale, staining granules are scattered in the cytoplasm but tend to localize near the cytocentrum and mitochondria. Cholesterol and cholesterol esters cannot be demonstrated histochemically in the lymphocytes or their precursors

Present methods for the demonstration of lipids appear of little practical value in the study of lymphocytes in health and disease primarily because the histochemical methods for lipoproteins and phospholipids are as yet not adequate.

Enzymes

Alkaline Phosphatase. The lymphocytes of the blood exhibit a negative reaction for alkaline phosphatase following both the calcium phosphate[19, 43, 82, 95, 104] and azo dye[51, 81, 111] methods, while the lymphoid cells of lymphatic tissue exhibit a variable but weak reaction[21, 29, 33, 43, 61, 96, 104, 113]

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from different patients can be seen in comparing Figs 3-32 and 3-33 Sulfhydryls are localized in the cytoplasm and nuclear sap while the chromatin and nuclear membrane are essentially unstained The large vacuole seen in the cytoplasm of the lowest of the three lymphosarcoma cells represents a glycogen deposit

FIG. 3-34 Two lymphoblasts from acute lymphoblastic leukemia stained for sulfhydryl groups by the DDD method Considerable amounts of sulfhydryls are demonstrable in the cytoplasm and nucleoplasm of these cells

FIG. 3-35 Lymphocytes from chronic lymphocytic leukemia stained for nonspecific esterase (indoxyl acetate substrate) Indigo crystals localize in the cytoplasm of the four lymphoid cells

FIG. 3-36 Lymphosarcoma cells stained for nonspecific dehydrogenase, using nitro BT as the substrate Dehydrogenase is localized in the mitochondria.



FIGS 3 28 through 3 36 Histochemistry of leukemic lymphocytes (× 2000, reduced 25 per cent)

FIG 3 28 Lymphocyte from chronic lymphocytic leukemia stained by the periodic acid-Schiff method Note large number of PAS-positive cytoplasmic granules

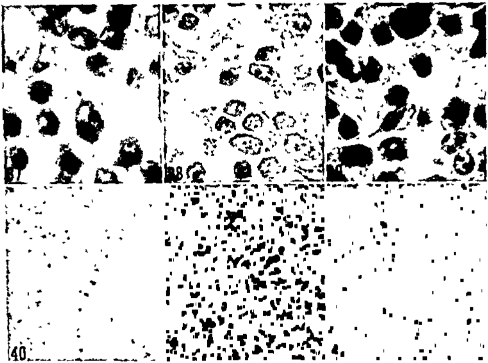
FIG. 3 29 Lymphosarcoma cells stained with the periodic acid Schiff method revealing a large number of PAS positive granules in their cytoplasm

FIG 3 30 Lymphoblast (*arrow*) from acute lymphoblastic leukemia stained by the periodic acid Schiff method PAS positive granules are very small and are generally less numerous than in normal lymphocytes

FIG 3-31 Two lymphocytes from chronic lymphocytic leukemia stained for protein-bound sulfhydryls by the DDD method Sulfhydryls localize to the cytoplasm and nu clear sap

FIG. 3-32 Two lymphosarcoma cells stained by the DDD method for protein bound sulfhydryls Sulfhydryls can be localized to the cytoplasm and nucleoplasm.

FIG. 3 33 Considerable variations in sulfhydryl content of the lymphosarcoma cells



Figs 3-37 through 3-42 Histochemistry of sections of lymphoid tissue

Fig 3 37 Demonstration of protein bound sulfhydryls in the cytoplasm of the small lymphocytes and stellate reticulum cells in normal human lymph nodes DDD method with nuclei counterstained with methyl green (× 1800 reduced 25 per cent)

Fig 3 38 Demonstration of acid phosphatase activity in the cytoplasm of lymphocytes and stellate reticulum cells from normal human lymph node Nuclear staining has been considered to be nonspecific Gomori acid phosphatase method with prolonged incubation time (16 hours). (× 1800, reduced 25 per cent)

Fig 3 39 Alkaline phosphatase is demonstrable in small amounts in the cytoplasm of the lymphocytes and stellate reticulum cells of normal human lymph nodes Nuclear staining has been considered as nonspecific Gomori alkaline phosphatase method with prolonged incubation time (16 hours) (× 1800, reduced 25 per cent)

Fig 3-40 *Localization of beta-glucuronidase in the lymphoid cells of rat spleen* Darker staining cells represent macrophages Method of Seligman *et al* (× 300 reduced 25 per cent)

Fig 3 41 Rat lymph node stained for alkaline phosphatase by the Gomori method Incubation time 14 hours (× 300, reduced 25 per cent)

Fig 3-42 Rat lymph node stained for alkaline phosphatase by the Gomori method 2 hours following 600 r total body x radiation Note the marked decrease in reactivity of the lymphoid cells following radiation Incubation time 14 hours (× 300, reduced 25 per cent)

With short incubation times the lymphocytes (one of thirteen patients) with chronic lymphocytic leukemia revealed increased phosphatase reactions, while the lymphocytes in acute lymphocytic leukemia were essentially lacking in enzymatic activity.[57] With prolonged incubation times, 14 to 18 hours, the lymphoid cells in normal human lymph nodes (Fig 3-39) and spleen show a positive reaction with the calcium phosphate method[1,3] A nonspecific nuclear reaction is most prominent with this technique,[1,99] while the cytoplasm of the lymphocytes and lymphoblasts shows a weak reaction for alkaline phosphatase activity.[1] Lymphoid cells from lymph nodes exhibit no increase in phosphatase activity in lymphosarcoma or chronic lymphocytic leukemia with the Gomori method.[1] The interpretation of positive reactions of lymphoid cells as indicating alkaline phosphatase must be guarded. 5-Nucleotidase activity is demonstrable in the cells of the germinal centers of normal human lymph nodes, but these investigators were unable to demonstrate alkaline phosphatase activity by either the Gomori method or the azo dye procedures.[23]

The alkaline phosphatase activity of normal lymphocytes is markedly altered in the lymph nodes (Figs. 3-41, 3-42) and spleen of rats within two hours following total body x-radiation at which time there is a decrease in phosphatase activity of the lymphocytes as measured by the calcium phosphate method of Gomori.[3]

Acid Phosphatase. Some lymphocytes in the normal blood have yielded a positive reaction for acid phosphatase localizing in their cytoplasm near the cytocentrum following the lead phosphate method of Gomori[82] When this reaction is applied to lymph nodes by using prolonged incubation times (Fig 3-38), a variable reaction is obtained in the lymphoid cells, with lymphoblasts exhibiting a somewhat more intense cytoplasmic reaction than the more mature lymphocytes[1] However, most investigators[23] have failed to demonstrate acid phosphatase activity in the lymphocytes of the blood and lymphatic tissue Nonspecific nuclear staining may be observed in the lymphocytes in both the blood and lymphatic tissue following the Gomori procedure The positive reaction of lymphoid cells to the Gomori method must be interpreted with caution.

Lymphocytes present in guinea pig spleen are negative following the azo dye methods for acid phosphatase employing alpha-naphthyl phosphate as the substrate,[25] but with 6-benzoyl-2-naphthyl phosphate splenic tissue is stained although germinal centers fail to react unless incubation times are prolonged[88] Acid phosphatase activity could not be demonstrated in normal human lymph nodes when the 6-benzoyl-2-naphthyl phosphate substrate was employed.[23]

Esterase and Lipase. Lipase and esterase activity has not been demon-

dase activity with the method employed.[106]

Dehydrogenases. Succinic dehydrogenases is demonstrable in the cytoplasm of the lymphocytes in normal blood[105, 104] and lymphatic tissue.[15, 16, 73, 89, 98] Although other investigators have failed to demonstrate dehydrogenase activity in lymphatic tissue,[24, 70] the lymphocytes exhibit one to several fine, purplish granules in their cytoplasm following incubation in buffered neotetrazolium.[104] Nitro BT appears to be a more sensitive substrate and permits better localization of dehydrogenase activity in the lymphocytes than is obtained with neotetrazolium in the blood and lymphatic tissue (spleen).[73] In lymphatic tissues (lymph nodes and tonsils) the neotetrazolium reaction reveals dehydrogenase activity in about half of the lymphocytes.[105] The nitroneotetrazolium chloride (NNT) substrate yielded negative reactions when applied to the spleen even after using an extended time in incubation.[75] Marked variations in dehydrogenase activity of the lymphocytes in chronic lymphocytic leukemia have been observed.[105] The percentages of reactive lymphocytes in ten cases of chronic lymphocytic leukemia were as follows: one case with 10 per cent, six cases with 2 to 5 per cent, two cases with over 90 per cent, and one case with no lymphocytes exhibiting dehydrogenase activity.[105] Dehydrogenase activity demonstrated with 2,3,5-triphenyl tetrazolium chloride (TTC) as the substrate reveals no appreciable activity with only a few fine formazan crystals in the lymphocytes in the follicles and pulp of the normal lymph node.[16] In contrast to the normal cells, the lymphocytes in lymphocytic leukemia and lymphosarcoma reveal active reduction of formazan, indicating increased enzyme activity in these abnormal cells.[16] Lymphocytes in the perifollicular and pulp areas of the normal spleen exhibit several fine and coarse formazan granules in their cytoplasm while the immature cells of the nodules exhibit less dehydrogenase activity.[15] The endogenous dehydrogenase activity of the lymphocytes, particularly in the perifollicular areas, is increased in hypersplenism.[15] By applying a modified nitro BT procedure to normal and leukemic lymphocytes of the blood, dehydrogenase activity has been observed in the lymphoid cells of lymphosarcoma (Fig. 3-36) and to a lesser extent in lymphoblastic leukemia.[9] Normal lymphocytes (Fig. 3-27) and those of chronic lymphocytic leukemia only rarely exhibited a positive reaction with this procedure. The dehydrogenase activity was localized to the mitochondria.[9] Histochemical demonstration of dehydrogenase activity is a relatively new field. The wide variety of techniques and the use of tetrazolium salts in the study of lymphoid cells must be evaluated in the light of newer and more sensitive methods.

Cytochrome Oxidase. Between 5 and 20 granules reacting with indophenol blue are demonstrable in the cytoplasm of the lymphocytes in the

strated histochemically in the lymphocytes by either the older Tween methods[44] or the more recent azo dye procedures employing alpha-naphthyl acetate,[23, 77] beta-naphthyl acetate,[72] napthol AS acetate,[23] napthol AS chloroacetate,[45] and 5-bromo-indoxyl acetate as substrates.[77] Cytochemically only minimal amounts of esterase activity has been ascribed to the lymphoid cells in lymphatic tissue.[33, 34] However, using the indoxyl acetate substrate with vital preparations, esterase activity can be seen in the cytoplasm of the lymphocytes (Fig. 3-26) as deposits of indigo blue crystals which tend to localize near mitochondria.[7] Larger lymphocytes may exhibit a somewhat greater esterase activity with this method than the small lymphocytes in normal blood.[7] Esterase activity has also been demonstrated in the cytoplasm of the lymphoid cells from patients with chronic lymphocytic leukemia (Fig 3-35), lymphoblastic leukemia, and lymphosarcoma.[8] However, significant differences in the reactivity of these abnormal lymphocytes and normal lymphocytes was not observed with this technique.[8] By using a modified procedure and napthol AS acetate as the substrate, esterase activity has been demonstrated in the cytoplasm of lymphocytes in fixed films in both normal blood and lymphocytic leukemia [107] Lymphocytes fail to stain following methods for the demonstration of cholinesterase.[35, 115] Nonspecific esterase activity increases in the lymphatic tissue of the rat during the regeneration period following x-radiation and decreases during the degenerative period [96] Improvement of histochemical methods for esterases may yield interesting results.

Phosphorylase. Phosphorylase activity could not be demonstrated in lymphocytes in normal blood films from a number of different species, including man,[102] or in the lymphoid cells of lymphatic tissue.[101, 102] However, the great majority of lymphocytes in chronic lymphocytic leukemia and lymphosarcoma exhibit phosphorylase activity, while the lymphoblasts seen in one case of acute lymphoblastic leukemia exhibited no phosphorylase activity [70] A positive correlation was noted between phosphorylase activity and the glycogen content in these cell forms, since glycogen was present in increased amounts in lymphosarcoma and chronic lymphocytic leukemia and decreased in acute lymphoblastic leukemia [70]

Beta-Glucuronidase. Beta-glucuronidase activity has been demonstrated in the lymphoid follicles of the spleen[39, 40, 91, 106] (Fig. 3-40) and tonsils but could not be definitely localized to the lymphoid cells [106] The cytoplasm of the round cells of the follicles exhibits the highest enzyme activity, particularly the cells at the periphery of the follicles [91] Considerable activity also has been described in the round cells of the splenic pulp[40, 91] although germinal centers are totally or partially unreactive [39] Lymphocytes as well as other leukocytes in blood and bone marrow films fail to exhibit beta-glucuroni

localization of various enzymes within the lymphoid cells, particularly the dehydrogenase, oxidative, and synthesing enzyme systems. Development of new techniques, wider application of present histochemical methods, and phase microscopy will continue to advance our knowledge of the structure, chemistry, and metabolism of the lymphocyte in both health and disease.

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blood[90] and lymphatic tissue[55] of man following the nadi reaction for cytochrome oxidase. These granules tend to localize on the mitochondria of the lymphocyte, indicating the presence of enzymatic activity in these structures.[55, 90] No quantitative differences in the G. nadi reaction were apparent between normal lymphocytes and those from patients with leukemia, lymphosarcoma, Hodgkin's disease, or tuberculous lymphadenitis.[55]

The specificity of the M. nadi method for cytochrome oxidase has been questioned and possibly may represent a fatty peroxide rather than an oxidative enzyme. The M. nadi indophenol reaction when applied to the lymphocyte has consistently been negative except in inflammatory exudates,[84, 85] in which cases the lymphocytes become very reactive and may exhibit up to 60 indophenol granules in their cytoplasm. This change in reactivity of the lymphocyte during the process of inflammation is correlated with an increase in sudanophilic and PAS-positive granules, peroxidase, and acid and alkaline phosphatase activity in these cells.[85]

Additional Enzymes. Attempts to demonstrate peroxidase activity in lymphocytes were consistently negative.[29, 69] Phosphamidase has been localized to the lymphoid tissue of the spleen[68] but could not be demonstrated in normal and hyperplastic lymph nodes.[23] With prolonged incubation times, all the cells of the spleen react positively for phosphamidase, and the mature lymphocytes exhibit a greater activity than lymphoblasts.[68] Beta-galactosidase has been localized to the lymphoid cords and follicles of the lymph nodes and in the red pulp of the spleen.[87] Ascorbic acid has recently been demonstrated in small amounts in lymphocytes present in normal peripheral blood.[27]

Antibodies. By using the fluorescent antibody techniques, "antibodies" have been demonstrated in small amounts in the lymphoid follicles of the lymph nodes and spleen in contrast to large amounts of fluorescent tagged antibodies observed in plasma cells.[60] These observations suggest that the lymphocytes apparently do not play a significant role in antibody formation.

SUMMARY

During the past few years we have added considerable to our knowledge concerning the chemical composition of the lymphocyte. It has become increasingly apparent that histochemical observations must be correlated with cellular morphology as seen in the living cell. Valid characterization and analysis of the morphology and histochemical structure of the cellular elements in leukemia must be derived from untreated patients. Studies concerning the effects of various therapeutic agents upon the chemistry of the leukemic cells have been only fragmentary. Perhaps the most promising area for further histochemical observations is in the demonstration and

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cised. The thinness of the sections used is of critical importance to the observer. A section of average thickness passing through the equator of a rounded-up cell contains considerably less than 1 per cent of the total volume of the cell. It is obvious that structures visualized in micrographs of this section are surely present in the cell, but the absence of any known or anticipated structure is not an indication that the cell does not possess it. The thin sections of electron microscopy also impose severe limitations on measurements of size, judgment of contours, and estimates of population densities. A discussion of these limitations, with special reference to blood cells, has been presented elsewhere (Reference 11, pp. 2-7). The proper interpretation of very high resolution electron micrographs may be attended by similar difficulty but for a distinctly different reason. In micrographs now routinely obtainable, resolution far surpasses section thinness, so that great difficulty may be encountered with proper judgment of size, shape, number, density, etc., when very small structures are being interpreted. This is because the optical situation in an electron microscope is such that the entire thickness of a section is equally clearly in focus. Depth of field exceeds section thickness in electron microscopy, whereas the reverse is true in light microscopy. The bearing of this phenomenon on interpretative problems, including a comparison with the optical situation in light microscopy, has been discussed in a previous paper (Reference 14, pp 242-246). Both thin sections and the unique optics of the electron miscroscope are responsible for characteristics in electron micrographs that are unfamiliar to light microscopists. Their fundamental nature is such that they point up the need for an essentially statistical approach to the analysis of protoplasmic ultrastructure. Final judgments may be considered safe only when based on a large number of electron micrographs These, in turn, must be interpreted with due regard for their inherent limitations as well as for the wealth of new detail they present

Special interpretative care should also be taken when comparing electron micrographs with light micrographs of dried smear preparations. In the latter all the contents of the cell remaining after drying and fixation are piled one on top of the other on the slide. The resultant patterns may be different from those of the thin sections of electron microscopy, where only a thin lamina of the cell is present Furthermore, the patterns visualized by the chromatic dyes of light microscopy differ from those visualized in electron micrographs, since the latter are largely an expression of the affinity of OsO_4 for ethylenic double bonds.[15]

The morphologic patterns familiar to the light microscopist cannot be expected to repeat themselves exactly in electron microscopy But the wealth of new information afforded by electron micrographs more than counter-

CHAPTER 4

ELECTRON MICROSCOPY OF THE LYMPHOCYTE*

FRANK N. LOW

The structure of blood cells, including the lymphocyte, has been observed with the electron microscope by numerous investigators in recent years.[1-10] An account of the ultrastructure of blood lymphocytes, somewhat more detailed than other contributions, may be found in the description of normal and leukemic human blood by the writer and James A. Freeman.[11] This chapter recapitulates the essential points of the original description, adds certain details recently clarified, and compares the ultrastructure of the lymphocyte with that of certain other blood cells. The electron microscopic blood picture in normal and leukemic human blood is interpreted with reference to the probable relationship of the lymphocytic series to the other developmental series of blood cells.

MATERIALS AND METHODS

Peripheral human blood was used exclusively in this study. Samples were obtained by the Freeman method[11] which is based on careful use of non-wetting agents and refrigeration. This method does not add any foreign substance to the blood prior to fixation. The remainder of the technique, including buffered OsO_4 fixation,[12] was empirically modified to facilitate handling of the buffy coat but did not differ in any essential respect from the routine preparatory techniques of electron microscopy described by Farquhar.[13]

Electron micrographs differ from light micrographs in certain ways that may lead to significant interpretative errors, unless proper caution is exer-

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files of *endoplasmic reticulum* are sparse compared to the larger number in certain other cells (monocytes) but are always present to some extent. Other formed elements, such as *compound vacuoles, Golgi zone, centrioles, azurophilic granules,* and *lipid granules,* may or may not be present in the plane of section.

CYTOPLASM

Lymphocyte *mitochondria* are large (Fig. 4-4) with sausage-shaped forms predominating (Reference 11, pp. 102-107). The clearly double cristae are characteristically oriented at right angles to the long axis of the mito-



FIG. 4-3. Lymphocyte; normal human blood. Endoplasmic reticulum (*er*) and mitochondria (*m*) are visible. In Figs. 4-1, 4-2, and 4-3, the 2 nucleoplasmic densities, light and dark, are arranged in the essentially patternless fashion that characterizes lymphocytes, and their well-developed contrast indicates mature cells. The number of mitochondria (*m*) in these particular cell sections are smaller than usual (see Fig. 4-4). (× 8500)

chondrion. In the round or oval forms, the cristae are usually irregularly arranged or even absent, a circumstance suggesting that the plane of section passes at nearly right angles to the long axis. The mitochondria may be located anywhere in the cytoplasm. Occasionally a cluster of them is observed around the Golgi zone. Mitochondrial ultrastructure in the lymphocyte conforms to the pattern well recognized to be characteristic of mitochondria in general.[15]

The *endoplasmic reticulum* in lymphocytes is visible as small, round, oval or sometimes irregular profiles (Figs. 4-4, 4-5). This form is characteristic of all blood cells except plasma cells. The profiles are scattered at random throughout the cytoplasm and each is composed of a single dense membrane which encloses a clear area.[14] Near the surface of the cell, the profiles are frequently in linear relationship to each other and are closely associated

balances interpretative difficulties. The gradually accumulating knowledge of ultrastructure in lymphocytes and other blood cells[1 11] may now be collated with the classic evidence obtained from the light microscope[16, 17] to formulate new concepts of hematologic organization.

RESULTS

Lymphocytes in electron microscopic preparations may present considerable variations in general appearance (Reference 11, pp. 64-77), but a

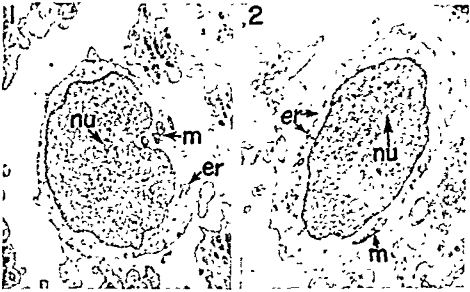


FIG 4 1 Lymphocyte, normal human blood The generally clear cytoplasm and sparse profiles of endoplasmic reticulum (*er*) and nucleolus (*nu*) are all characteristic. (× 8600)

FIG. 4-2 Lymphocyte, normal human blood. A nucleolus (*nu*) is visible, and profiles of endoplasmic reticulum are visible (*er*) in the generally clear cytoplasm (× 9500)

distinct structural type is recognizable (Figs 4-1, 4 2, 4-3, 4-10). The *nucleus* is often large and well rounded, but slight variations of contour may become exaggerated due to the thinness of the section The nucleoplasm shows two different densities (Figs 4-10, 4-11), the dark and the light areas being mixed haphazardly with each other without the definite patternization that occurs in other mature blood cells (Reference 11, pp. 45, 57, 78) One or more *nucleoli* may be present, depending on the plane of section. The outstanding characteristic of the *cytoplasm* is its clarity, which is conspicuous when compared to that of other cell types. Among the formed elements the rather large *mitochondria* are conspicuous The small circular or oval pro-

with the outfoldings of cytoplasm that characterize many lymphocytes prepared by this technique.

Compound vacuoles are a notable feature of lymphocytic ultrastructure (Figs. 4-5, 4-6), although occurring in other blood cell types (Reference 11, p. 117), notably blood monocytes, and in the rat spleen.[19] They consist essentially of a variable number of small vacuoles contained in a larger one. Outside of the larger vacuole, there are frequently clusters of smaller ones which are indistinguishable from the small ones inside. The vacuoles in the complex are structurally separate. The number of small vacuoles

7



FIG. 4-7. Detail of lymphocyte, normal human blood. The Golgi zone (G) consists of flattened sacs, smaller rounded profiles, and a few large vacuoles. Within the concavity of the Golgi zone is the relatively lucid and nearly structureless centrosphere (*cs*). In the center of the latter is a centriole (*cl*), here cut in cross section. The 2 components of the nuclear membrane (*nm*) are closely approximated in some areas and more widely separated in others. (× 41,000)

within the parent vacuole in a single section vary from only one or two to a conglomerate so densely packed that recognition of the complex as a compound vacuole may be difficult.

The "*small particulate component*" of Palade[20] is readily recognizable in blood lymphocytes (Figs. 4-4, 4-5, 4-6), but the granules do not show an affinity for the endoplasmic reticulum. This situation is characteristic for all of the blood cells except plasma cells (Reference 11, pp. 112, 322).

The *Golgi zone, centrosphere,* and *centrioles* are so intimately related in lymphocytes that they merit description together. The established concept of light microscopy for large lymphocytes[17] is that two centrioles are surrounded by a cytocentrum which in turn is surrounded by the Golgi zone

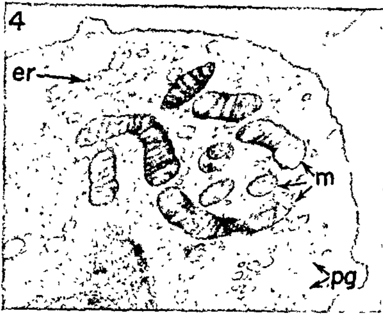
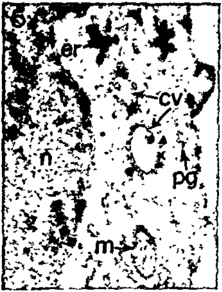


FIG. 4-4 Detail of large lymphocyte; normal human blood The large mitochondria (*m*) are conspicuous. The circular profiles probably represent cross sections Numerous profiles of endo plasmic reticulum (*er*) and randomly scattered Palade granules (*pg*) may be seen (× 21,000)



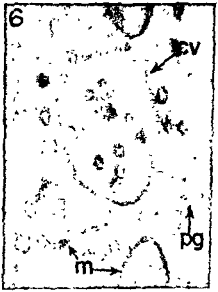


FIG 4-5 Detail of lymphocyte, normal human blood Numerous profiles of endoplasmic reticulum (*er*) are visible above Each of the 2 compound vacuoles (*cv*) has a cluster of small vacuoles around it (× 29,000)

FIG 4-6 Detail of lymphocyte normal human blood This compound vacuole (*cv*) is typical, with a cluster of vacuoles surrounding it and numerous small profiles within (× 53,000)

with the outfoldings of cytoplasm that characterize many lymphocytes prepared by this technique.

Compound vacuoles are a notable feature of lymphocytic ultrastructure (Figs 4-5, 4-6), although occurring in other blood cell types (Reference 11, p 117), notably blood monocytes, and in the rat spleen.[19] They consist essentially of a variable number of small vacuoles contained in a larger one. Outside of the larger vacuole, there are frequently clusters of smaller ones which are indistinguishable from the small ones inside. The vacuoles in the complex are structurally separate. The number of small vacuoles



Fig. 4-7. Detail of lymphocyte, normal human blood The Golgi zone (*G*) consists of flattened sacs, smaller rounded profiles, and a few large vacuoles Within the concavity of the Golgi zone is the relatively lucid and nearly structureless centrosphere (*cs*) In the center of the latter is a centriole (*cl*), here cut in cross section. The 2 components of the nuclear membrane (*nm*) are closely approximated in some areas and more widely separated in others (× 41,000)

within the parent vacuole in a single section vary from only one or two to a conglomerate so densely packed that recognition of the complex as a compound vacuole may be difficult

The "*small particulate component*" of Palade[20] is readily recognizable in blood lymphocytes (Figs. 4 4, 4-5, 4-6), but the granules do not show an affinity for the endoplasmic reticulum. This situation is characteristic for all of the blood cells except plasma cells (Reference 11, pp 112, 322)

The *Golgi zone, centrosphere,* and *centrioles* are so intimately related in lymphocytes that they merit description together The established concept of light microscopy for large lymphocytes[17] is that two centrioles are surrounded by a cytocentrum which in turn is surrounded by the Golgi zone

In small lymphocytes the Golgi zone is usually to one side of the centrosphere. In fortuitous sections (Fig. 4-7) the Golgi zone is demonstrable as a series of flattened sacs, circular or oval profiles of vacuoles, and larger vacuoles with clear center and indistinct outlines. The flattened sacs have a curvature which partly encircles the centrosphere. The centrosphere itself is generally lucid but of irregular density and usually contains no clearly defined structures except the centrioles. Occasionally one (Fig. 4-7) or, more rarely, two centrioles may be observed within the centrosphere The centriole, when visualized in cross section, is seen to be composed chiefly of a circlet of nine rodlets. The electron microscopy of the centrioles, centrosphere, and associated Golgi zone in blood cells has been described by others.[21]

Azurophilic granules and granules or *droplets of high lipoid content* are both recognized to be inconstant inclusions of lymphocytes[16] The former are only infrequently seen in electron microscopic preparations (Reference 11, p. 121). They are composed of a moderately dense, homogeneous core which is surrounded by a lucid zone On the outside there is an apparently membranous covering of about the same density as the core. Granules with high lipoid content are very variable in size, shape, and density. Often an inclusion within the granule occupies more than half its area in the micrograph and may be either denser or more lucid than the core Occasionally there may be lamination (Reference 11, p. 123)

NUCLEUS

The *nuclear membrane* of lymphocytes has no special features, conforming to the pattern of ultrastructure characterizing cells in general (References 11, p 119, and 22). It is double, with each component tracing an irregularly wavy course. *Nuclear pores* are present and seem to be more readily demonstrable in the larger cells The *nucleoli* of lymphocytes possess no structural organization special to the lymphocyte[23] Close examination of nucleoli reveals a structureless background somewhat denser than the nuclear substrate elsewhere (i e, in areas of the nucleus not containing a nucleolus). A heavy population of the dense granules that are characteristic of nucleoplasm fixed by the buffered OsO_4 technique completes the picture.

Nucleoplasmic density in these OsO_4 preparations is worthy of special notice, since its patternization is a most useful index of the stage of development of the cell (Reference 11, pp 261, 281). The dense granules of the *nucleoplasm* are irregularly distributed against an amorphous background which itself may be subject to considerable density variation, especially in mature cells In very young cells such as the myeloblasts, monoblasts, and lymphoblasts of the peripheral blood of leukemic patients, both

nuclear granules and substrate are so evenly distributed that the nucleus as a whole is of even density (Fig. 1-8). As development progresses, changes in granular concentration and density of substrate produce dark and light areas of nucleoplasm (Fig. 1-9). Patterns characteristic of the different cell types are formed, the greatest contrast between light and dark areas being attained in the mature cells (Figs. 4-10, 1-11).

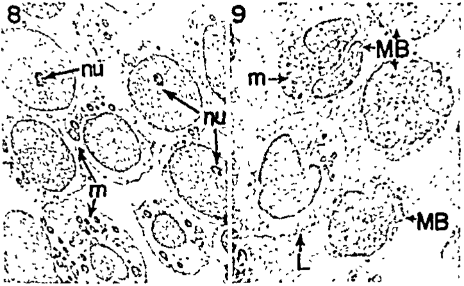


Fig 4 8 Lymphoblasts, lymphocytic leukemia Note the even density of the nucleoplasm everywhere except at the nucleoli (*nu*) The cytoplasm is extremely lucid and the mitochondria (*m*) conspicuous (× 5500)

Fig 4-9 Monoblasts and cell of lymphocytic series, monoblastic leukemia The monoblasts (*MB*) have dark cytoplasm with mitochondria (*m*) The nucleoplasm has begun to differentiate chiefly as a thin dark band inside the nuclear membrane The cytoplasm of the cell at *L* is very light, and this identifies it as a member of the lymphocytic series (× 6100)

The *lymphocytic and monocytic series of development* can readily be recognized (Figs 4-8, 1-9) in the peripheral blood pictures of the leukemias involving these two developmental series (Reference 11, pp 284-293) The lymphocytic series is characterized by very lucid cytoplasm that is generally clear except for the large mitochondria that are often the only conspicuous contents (Fig 4-8) The nucleoplasm differentiates, as the cell passes from lymphoblast to lymphocyte, into light and dark areas These areas, however, are arranged in an essentially patternless fashion (Figs 4-1, 1-2, 4-3, 4-10), without noticeable affinity of the denser portion for the nuclear membrane The monocytic series can be readily distinguished from the lymphocytic

series because it is characterized by dark cytoplasm having a generally speckled appearance. The latter is largely traceable to abundant endoplasmic reticulum and to small mitochondria. The nucleoplasm begins its differentiation in the monoblast as a narrow dense band just inside the nuclear membrane (Fig 4-9) The mature pattern is characterized by this band, now wider, and a few dense areas scattered deeper within the nucleus (Fig 4-11). Very marked distinction between the lymphocytic and mono-



FIG 4 10 Lymphocyte, normal human blood The cytoplasm is clear and the nucleoplasm has 2 distinct densities which are essentially patternless in their distribution Compare with the monocyte in Fig 4 11 (× 9500)

FIG 4 11 Monocyte, normal human blood The speckled cytoplasm contains numerous small mitochondria and profiles of endoplasmic reticulum (*er*) The nucleoplasm has 2 distinct densities the darker of which forms a distinct narrow band under the nuclear membrane, with a few dense patches deeper in the nucleus The well-developed nucleoplasmic densities of Figs 4 10 and 4-11 indicate that the cells are mature (× 8600)

cytic series is afforded by blood in monoblastic leukemias which contains a few members of the lymphocytic series (Fig 4-9) (Reference 11, pp 285, 289, 293, 295) Each of these two developmental series in the human leukemias is readily identifiable with its own end product, the mature monocyte or lymphocyte of normal peripheral blood The chief characteristics of mature cells of these two types are summarized below.

Cells possessing all or most of the above characteristics are very readily identified as either lymphocytes (Fig 4-10), or monocytes (Fig 4-11) This

	Lymphocyte	*Monocyte*
Nucleoplasm:	dark areas distributed in essentially patternless fashion	dark band inside of nuclear membrane, some dark areas within nucleus
Cytoplasm.	clear appearance	speckled appearance
Mitochondria:	large, moderate number	small, numerous
Endoplasmic reticulum:	small, round or oval profiles, sparse	small, round or oval profiles, very numerous

holds true regardless of the dimensions of the cell. A large lymphocyte is clearly distinct from a monocyte. But some agranulocytes in normal, human blood possess morphologic characteristics that make uncertain their identification as either monocytes or lymphocytes. For example, the cell in Figure 4-12 has an indifferent pattern of nucleoplasmic differentiation and large mitochondria resembling those of a lymphocyte. But its cytoplasm has many profiles of endoplasmic reticulum and is darker than lymphocytic cytoplasm, both characteristics suggesting a monocyte Observation of many agranulocytes of uncertain identity has failed to reveal any pattern governing the mixture of confusing characteristics. Mitochondria, endoplasmic reticulum, general cytoplasmic characteristics, and nucleoplasmic patterns may be combined in a single cell in the forms characteristic of either cell type so as to produce all possible varieties of structural intergrade.

Some agranulocytes in normal peripheral blood possess nucleoplasm that is either uncertainly differentiated (Fig 4-13) or of even density throughout (Fig. 4-14) The evidence of progressive nucleoplasmic differentiation afforded by the leukemias, notably lymphatic leukemia, (Figs 4-8, 4-15) seems to justify the classification of these cells as lymphoblasts

SUMMARY

The evidence now available from the electron microscopy of normal and leukemic human blood[11] may be used to compare the lymphocyte and its developmental series to the other blood cells and their developmental series In brief, the evidence from the human leukemias indicates that the parent cell of the bone marrow usually called a myeloblast (stem cell) and the parent cell of the monocytic series, the monoblast, are morphologically indistinguishable from each other under the electron microscope. But the parent cell of the lymphocytic series, the lymphoblast, is always readily identifiable as such There is never any danger of confusing it with any

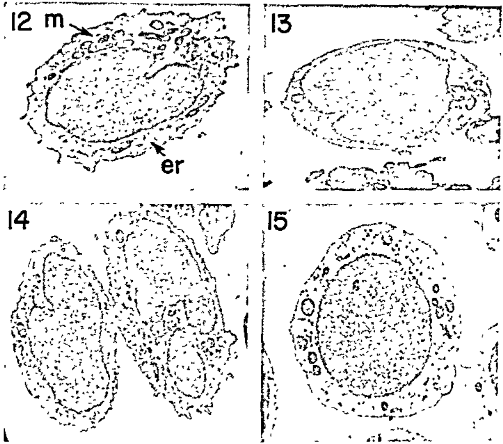


FIG 4 12 Unidentified agranulocyte This "lymphocyte monocyte intergrade" cannot be certainly identified as either cell type The large mitochondria suggest a lymphocyte, but the speckled cytoplasm containing numerous profiles of endoplasmic reticulum resembles monocytic cytoplasm The nucleus is noncommittal with some tendency for the dense nucleoplasm to collect under the nuclear membrane But nucleoplasmic differentiation does not show clearly, and the cell may be immature (× 6000)

FIG 4 13 Lymphoblast, normal human blood The nearly even density of the nucleoplasm indicates immaturity Compare with the mature lymphocyte in Fig 4 10 and the leukemic lymphoblast in Fig 4-15 (× 6000)

FIG 4-14 Lymphoblasts normal human blood The even density of the nucleoplasm suggests that these cells are even younger than the cell in Fig 4-13 Compare also with Fig 4-10 and 4 15 (× 6000)

FIG 4-15 Lymphoblast lymphatic leukemia The even density of the nucleoplasm and the extremely lucid cytoplasm are both characteristic of lymphoblasts In the lymphocytic series the early blastic forms tend to be noticeably less dense in both nucleus and cytoplasm than mature cells Compare with Figs 4-10, 4-13, and 4 14 (× 6500)

of the other cell types, regardless of their stage of development. In normal peripheral blood, although there are cells that cannot be positively identified as either lymphocyte or monocyte, many cells are present with lymphocytic characteristics that indubitably connect them with the parent lymphoblast. The few plasma cells in normal peripheral blood are, in electron microscopic morphology, much closer to monocytes than to lymphocytes, and "monocyte-plasma cell intergrades" have been observed. The weight of the electron microscopic evidence now available argues strongly that, apart from all other developmental series of blood cells and their derivatives, the lymphocytic series stands alone.

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CHAPTER 5

LYMPHOCYTIC LIFE SPAN AND TURNOVER

JESSE L. BOLLMAN

Lymphocytes are formed in several sites in the body from which they migrate into the blood and lymph The life span of the lymphocytes would be the total number of body lymphocytes divided by the average of the numbers formed in each tissue each day Another method for the determination of life span is the administration of labeled lymphocytes with subsequent determination of the rate of disappearance of the labeled lymphocytes from the blood, lymph, and tissues A third method involves the drainage of lymphocytes from the body via a lymphatic fistula and the calculation of the decrease of the lymphocytes in the blood There are modifications and combinations of the above methods Each method, however, has several complicating factors which, in addition to the technical difficulties, include different rates of formation and destruction of lymphocytes in the different tissues, the circulation of lymphocytes from the blood, lymph, and tissues, and the reutilization of labeled material, either by division of the lymphocytes or reutilization in new lymphocytes At the present time we can only give an approximation of the life span of lymphocytes, and even on the approximation there is a diversity of opinion

The bone marrow is most rich with lymphocytes Yoffey and Courtice[1] counted 300,000 lymphocytes per cubic millimeter from guinea pig bone marrow and estimated that the total number was between ten and twenty times that of the blood By counting the number of cells in visible phases of mitosis, Widner *et al*[2] calculated, on the basis of 25 minutes' duration for mitosis as observed, that the cells of the bone marrow of the rat were completely renewed in from 1 4 to 1 4 days.

Yoffey,[3] Kindred,[4] and I[18] have observed that a large number of labeled

lymphocytes injected into the blood find their way into the bone marrow, and it is believed that some of these divide to form new cells in the bone marrow.

The lymphoid tissue of the mucosa of the gastrointestinal tract contains about three times as many lymphocytes as are present in the blood. Lymph from nonlymphoid tissue contains 200-800 lymphocytes as compared with 40,000–50,000 in intestinal lymph. Since the volume of intestinal lymph that enters the thoracic duct in 24 hours is about twice that of the circulating blood volume, about 20 times as many lymphocytes enter the blood from the intestinal lymphatics as are present in the blood at any one time. These lymphocytes, with those from the bone marrow, indicate a replacement of blood lymphocytes in less than one hour.

External drainage of the thoracic duct or intestinal lymphatics of rats produces a loss of 30–50 million lymphocytes in one hour. After one day's drainage the lymphocyte count of the lymph has fallen to about half of the original figure, and by the end of four days only 8–10 million lymphocytes appear in the lymph, although the volume is well maintained. At the same time the lymphocytes of the blood are markedly decreased. This observation alone indicates that there must be a large recirculation of lymphocytes and at the same time a rapid formation of new lymphocytes. Reinfusion of the lymph or the lymphocytes has until recently failed to replace the decrease observed. By meticulous care in the preservation of the lymphocytes to be injected, Gowans[5] and I[18] have been able to maintain a steady flow of lymphocytes in lymph by reinfusion of the withdrawn lymphocytes and even further to increase the number of lymphocytes in the lymph by the infusion of additional lymphocytes obtained from lymph of other rats. If lymphocytes labeled with P^{32} or with Cr^{51} were infused into the blood, these began to appear in the intestinal lymph within two hours. Killed lymphocytes did not appear in the lymph in appreciable numbers but accumulated largely in the spleen and liver with only traces appearing in other tissues. Following the injection of carefully prepared lymphocytes which are still motile, the number taken up by the spleen and liver is much less than with killed lymphocytes but is still sufficiently large to indicate that circulating lymphocytes also reenter these tissues. The lung also accumulates killed lymphocytes, but only a relatively few of the live lymphocytes are removed by the lung. Mitotic counts of the lymphoid follicles of the spleen indicate that these replace themselves once in from 6 to 12 days, but no account has been taken of the lymphocytes which reenter this organ.

The thymus supplies considerable numbers of lymphocytes to the blood. By studying mitotic figures in the thymus, Kindred[4] has calculated that most of the blood lymphocytes in young rats are supplied by the thymus

This and labeling techniques indicate a replacement of thymic lymphocytes in rats once every two and one-half days. Labeled circulating lymphocytes also reenter the thymus in considerable numbers. Similar studies of lymphocytes in lymph nodes indicate a rapid mitotic replacement of lymphocytes Circulating labeled lymphocytes also reenter the lymph nodes in common with all lymphoid tissue.

Transfused lymphocytes do not remain in the blood but are there for a short time and disappear in from one to three hours. The disintegration of lymphocytes in the blood is not great, as lymphocytes added to blood in vitro remain motile for much longer times.

Only a few lymphocytes are excreted into the lumen of the intestine, as very few are found in the surface layers of the intestinal mucosa as compared to the larger numbers found in the deeper layers. Transfused P^{32}-labeled lymphocytes from the thoracic duct leave the blood rapidly, and most are found in the lymphoid tissue, depending on the viability of the lymphocytes transfused. Van Dyke and Huff[6] radiated one member of parabiotic rats to destroy his circulating white cells and calculated the white cells transfused to this rat from his otherwise normal parabiotic partner. The average survival time of the granulocytes in the blood of the recipient rat was 23 minutes and that of the lymphocytes was 170 minutes Farr[7] found that labeled autogenesis lymphocytes from lymph nodes of the rabbit disappeared from the blood within 90 minutes. Transfused lymphatic leukemia cells leave the blood in about one hour White[8] transfused blood from men who had received Atabrine (which labeled the leucocytes) into recipients and found that the leucocytes had disappeared from the arterial blood in from 30 to 90 minutes. Gowans[5] recovered 80 per cent of the lymphocytes transfused into the blood from the lymph of the thoracic duct Certainly there is a large and rapid migration of lymphocytes from the blood to the lymphoid tissues and to the lymph. The number of circulations seem adequate for the individual lymphocyte to visit most of the lymphatic tissue of the body. The daily output of lymphocytes in the thoracic duct of a dog weighing 10 kg. was found to be 5 078 million daily and that of man was estimated to be 35,550 million each day

From the data available Osgood[9] has calculated that the life span of the lymphocyte in the human is 100 days Less definite information is available for the large and medium lymphocytes The many factors involved in calculating the life span of lymphocytes and the lack of precision in the calculation of each of these would make this estimate an approximation

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CHAPTER 6

ROLE OF THE LYMPHOCYTE IN INFLAMMATION

JOSEPH C. SIERACKI AND JOHN W. REBUCK

In the lesions of chronic inflammation, lymphocytes and plasma cells form part of the characteristic inflammatory exudate. The retention of the characteristic lymphocyte morphology in such lesions as perivascular cuffing in paresis (Fig. 6-1) and about the periphery of various granulomas (Fig 6-2) is so obvious that no major dispute exists as to the origin of these cells in these so-called small, round cell infiltrations. In such lesions, since there is little histologic evidence of any phagocytic powers among the lymphocytes, some pathologists have questioned whether lymphocytes can ever be phagocytic

The lymphocytes in inflammation are concerned with a number of known interrelated functions Some of these are outlined in Table 6-1 The immunologic, trephocytic, and other functions will be discussed in detail by others in this monograph

Table 6-1. Some Functions of Lymphocytes in Inflammation

I Phagocytosis
1 Important mobile source of motile macrophages
2 Rich in adenosinase—destroy products of protein catabolism
3 Acid active proteoses—removal fibrin rbc, etc
II Immunologic Phenomena
Produce antibodies, transfer hypersensitivity, etc
III Trephocytic Functions
Labile protein and nucleic acid depots
IV Cytopoietic Functions
Source of other cells (e g plasma cells fibroblasts) as well as other lymphocytes

PHAGOCYTOSIS

Phagocytosis is an important function of exudate cells. No lesser an investigator than Metchnikoff in 1888[1] first demonstrated that blood lymphocytes in experimental animals migrated to the areas infected with tubercle bacilli, gradually hypertrophied, and formed macrophages and epithelioid cells. Although more positive information has been published to support this thesis, rather than to deny it, many textbooks of pathology have ex-

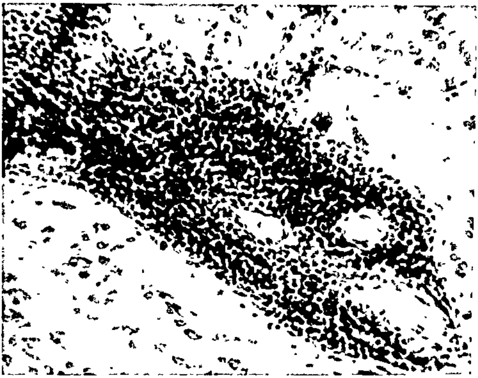


FIG 6 1 Lymphocytic infiltrations in chronic parenchymatous syphilitic encephalitis (paresis) Nissl's stain (× 375)

cluded the hematogenous (lymphocytic) macrophages as part of our general defense system of cells This can be traced in part to the so-called German school of thought typified by Aschoff In 1913 he and Kiyono published their work[2] on the "large mononuclears" and did not mention the lymphocyte because, under the conditions of their experiments, it did not store vital dyes

Another objection was the belief that lymphocytes were incapable of ameboid motion and, therefore, could not migrate from the vessels into

areas of inflammation. These objections have been disproved by numerous investigators and are no longer tenable. The literature on these views as well as the potentialities of the lymphocyte has been reviewed recently by Rebuck and colleagues.[3, 4]

Some of the prerequisites of readily mobilized sources of potential macrophages are:

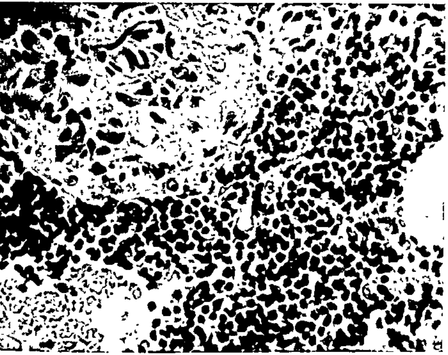


FIG 6 2. Subcutaneous granuloma of sarcoidosis with numerous lymphocytes at the periphery. Hematoxylin-eosin stain (× 560)

1. A source of *small* cells readily available for transportation to the inflammatory site
2. Access to a suitable means of transportation (blood stream)
3. The cells must be capable of motion
4. The cells must be capable of emigration from vessels and preferably show a positive chemotaxis to the inflammatory agent
5. The cells must be able to help destroy, contain, or "neutralize" the noxious stimulus so that healing can ensue

The neutrophilic leukocytes, numerically the most important members of the white cells in the peripheral blood, best fulfill the first four of these

conditions; however, their limited phagocytic powers are almost universally accepted They are called *microphages* because they do not readly phagocytize large particulate matter The lymphocytes, although they do not possess the power of positive chemotaxis, would numerically appear the next best possibility. This is indeed the case, and it has been proved many times. Of particular importance is the demonstration of this phenomenon in acute inflammation.

METHODS OF STUDY

The techniques used to study the changes within the first day of the inflammatory response should allow samples (biopsies) to be taken at frequent intervals during this phase of the reaction. Furthermore, such techniques should permit accurate comparison of the exudate cells with the leukocytes of the peripheral blood Direct microscopic observations, using transparent ear chamber technique, exteriorization of mesentery, etc, have been used to study these changes and have confirmed the emigration and motility of the lymphocytes Such direct techniques are of less value in later stages of the response when the number of exudate cells are markedly increased

The skin window and skin blister techniques have been used for such investigations The sequential biopsies may be studied with the same methods used for examining the leukocytes of the peripheral blood. The techniques and results have been recorded in detail elsewhere[5, 6] and will be summarized briefly in this report.

HUMAN SKIN WINDOWS

Control Series

Technique

The skin over the volar surface of the arm is shaved and cleansed with alcohol A 5 mm area of epithelium is scraped away with a sterile scalpel until the papillary layer of the corium is reached After sterile application of a drop of an inflammatory agent, the denuded corium is covered with a clean, sterile cover slip Then a strip of adhesive tape is applied to keep the cover slip in place At suitable time intervals the cover slip is replaced with a new one. The removed cover slips are rapidly air-dried and treated like a peripheral blood film

Results

The tissue cells, fibroblasts, and macrophages are the first to react in an area of inflammation The neutrophils are present at the site 30 minutes after the inflammatory stimulus.

At 6 hours (Fig. 6-3) the neutrophils are the most numerous of the exudate cells. They appear somewhat smaller and show varying signs of degeneration. The lymphocytes constitute about ⅓ to ¼ of the exudate

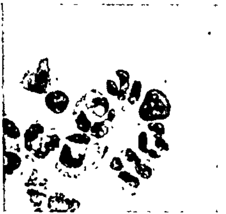


Fig 6-3. Two small lymphocytes and six neutrophils in the lesion of a control subject in the sixth hour of inflammation (× 900)

cells. The majority are small lymphocytes and range from 7 to 11 μ in diameter.

At 9 hours (Fig. 6-4) the lymphocytes constituted somewhat less than 50 per cent of the cells Their nuclear pattern showed a slight increase in parachromatin and an irregular outline of the nuclear membrane, often

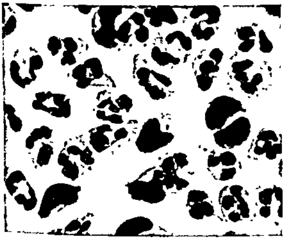


Fig 6-4 Six lymphocytes and numerous neutrophils from a lesion similar to that in Fig 6-3 in the ninth hour of inflammation in man (× 900)

with a cytocentric ridge or indentation The cytoplasmic membranes were irregular in outline, probably because they were fixed in a state of ameboid motion

At 12 hours (Fig 6-5) the majority of the exudate cells were lymphocytes The neutrophils had degenerated further, many of them measured 6 to

8 μ in diameter. The lymphocytes when tested for phagocytic activity were actively phagocytic with particles of India ink and had numerous vacuoles in their cytoplasm. The nuclear membrane became more ir

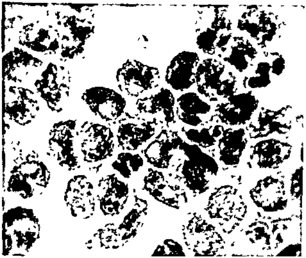


FIG 6-5 Thirty three lymphocytes and 3 neutrophils from a lesion similar to that in Fig. 6 3, in the twelfth hour of inflammation in man (× 900)

regular in outline and the nuclear pattern more porous or "open."

At 16 hours (Fig. 6-6) the lymphocytes showed absolute evidence of hypertrophy of nuclear and cytoplasmic mass Cell diameters ranged from



FIG 6 6 Hypertrophied lymphocytes from a lesion similar to that in Fig 6 3, in the sixteenth hour of inflammation in man (× 900)

11 to 14 μ in diameter There was division of large, coarse chromatin masses into smaller particles

At 25 hours (Fig 6-7) the cell diameters of the lymphocytogenous macrophages ranged from 11 to 17 μ There was an increase in nuclear size

as well. The nuclear membrane showed varying degrees of ridging or notching. The nuclear pattern is "more open" with an apparent increase of parachromatin. If observation of these cells had been started with the 25 hour stage, there would have been little to suggest their origin from the small lymphocyte.

Naegli Monocytic Leukemia Subject[4]

The patient suffered from chronic granulocytic leukemia (monocytic leukemia of Naegli) in which he had 6,000 fully mature monocytes per



FIG. 6-7. Hypertrophied lymphocytes from a lesion similar to that in Fig. 6-3, in the twenty fifth hour of inflammation in man. (× 900) *(Inset)* Monocyte from peripheral blood of volunteer at same magnification for comparison (× 900)

cubic millimeter of blood Their diameter was approximately 15 μ, and the majority (85 per cent) had prominent nuclear indentations as a tag Egg albumen was used as the inflammatory agent

At 7 hours the monocytes were present in moderate numbers and, except for polymorphous changes of their nuclei (thin strands), had no major morphologic alteration Some of the cells showed evidence of precocious phagocytosis

At 12 hours the monocyte was the predominant mononuclear cell They were further hypertrophied (16 to 18 μ in diameter) and had increased evidence of phagocytosis. When a control window at 12 hours and monocytic leukemia at the same interval were compared, the hypertrophied lymphocytes ranged from 8 to 12 μ while monocytes range from 14 to 18 μ in diameter, and the latter are more actively phagocytic Hypertrophied monocytic series during the first 13 hours of inflammation were approximately 6 μ larger than the comparable lymphocytic series

BLISTER: SUPRAVITAL METHOD

Technique

The blister technique permitted continuous direct observation of the lymphocyte macrophage transformation in supravital preparations. The blisters were produced on the volar surface of the forearm following the application of Chinese cantharides and a petrolatum mixture on a 1 5 cm circular area of the skin. The area was covered with adhesive tape to exclude air. In approximately 7 hours a 0.5 cm. blister is formed A mixture of egg albumen and neutral red solution in a physiologic saline solution was injected into the blister. Interval aspirations of a small amount of the blister fluid (via a #25 syringe needle) were studied as supravital preparations in a warm-stage enclosure.

Results

A lymphocyte from the exudate aspirate 7 hours and 55 minutes after the application of cantharides resembled the lymphocyte as seen in the peripheral blood and was characterized by its small size, round nucleus, scant cytoplasm, and the absence of neutral red vacuoles. This cell was maintained under direct, continuous observation, and 15 minutes later (8 hours and 10 minutes) the nuclear membrane reflected marked activity (represented by a serrated border) in the area of acquired neutral red vacuoles At an interval of 8 hours and 25 minutes (30 minutes after the initial observation of this cell), this lymphocyte developed a deep cleft or indentation of its nucleus, an increase in cytoplasmic mass, and 9 neutral red vacuoles.

Another lymphocyte from the aspirate of 11 hours and 20 minutes was observed Nineteen minutes later (11 hours and 39 minutes) it had ingested three albumen particles and had neutral red vacuoles

At 11 hours and 46 minutes, it was in contact with the neutrophilic bud but apparently did not ingest it There was some slight increase in cytoplasmic mass

At 11 hours and 51 minutes, the cell showed an indentation of the nucleus, ingested egg albumen, and from 7 to 15 neutral red vacuoles.

At 12 hours and 5 minutes (15 minutes after it was first observed), the nuclear mass had increased, and the nuclear membrane reflected marked activity The amounts of cytoplasm and neutral red vacuoles were also increased In the subsequent minute it developed a horseshoelike nucleus and a further increase in neutral red vacuoles The vacuoles had a rosettelike arrangement near the cytocentrum

At the 23 hour and 15 minute stage of the inflammatory response, motile macrophages of lymphocytic origin had the characteristics of a supravital "monocyte." These characteristics include a large cell body, a kidney bean-shaped nucleus, a neutral red rosette near the nuclear notch, a constant undulation of the cell body, and delicate pseudopodia extending in many directions

DISCUSSION

Lymphocytes play a dynamic role in the inflammatory response. Their known functions are roughly divisible into four major types: immunologic, trephocytic, cytopoietic, and phagocytic phenomena. These functions are often closely related, and this didactic classification is of necessity somewhat arbitrary. The experiments cited in this report are concerned primarily with those functions related to phagocytosis

Metchnikoff in 1888 first described the migration and hypertrophy of lymphocytes in rabbits and marmots infected with tubercle bacilli. In addition to his lectures in 1892, in which he demonstrated conclusively the lymphocyte ——→ macrophage transformation, six other confirmatory reports were published by 1900.

In 1902 Maximow published a 262-page review of his own experiments on inflammation. He confirmed the motility of lymphocytes and their emigration from vessels to the area of inflammation as well as their transformation into macrophages Up to 1950, over 70 similar confirmatory reports were published[3, 4] Many of these papers stress the importance of studying the inflammatory response in the early hours

The skin window and related techniques are simple, reliable methods of studying the early changes in humans and laboratory animals They confirm and extend the studies of Metchnikoff.

The tissue macrophages are the first cell to respond to the inflammatory stimulus when it is applied to connective tissue The neutrophils or their equivalent (pseudoeosinophils, etc) are usually the first of the hematogenous cells present at the inflammatory site. In man they are present within 1 hour and comprise the majority of the exudate cells for the first 10 to 11 hours in these experiments After the twelfth hour, they are present in decreased numbers and often show severe degeneration changes At 3 hours, only a few lymphocytes are present Six hours after the onset, they comprise about a third of the exudate cells From 2 to 14 hours and later, they are slightly hypertrophied and the predominant cell type At 14 hours the average cell diameters show only a light increase ranging between 11 and 13 μ At 21 hours the value is from 14 to 17 μ At 24 hours and later, they continue to increase their nuclear and cytoplasmic mass and become indis-

tinguishable from other connective tissue macrophages. The hypertrophy *of these cells is accompanied by morphologic changes.* If the study of the inflammatory process had begun after the 25 hour stage interval, it would be difficult to demonstrate lymphocytic origin of the macrophages

During the period of transformation, the lymphocyte develops an increase in cytoplasmic and nuclear mass. This is accompanied by evidence of increased phagocytic ability and a marked change in the appearance of the nuclear pattern. The nuclear membrane becomes increasingly irregular, the coarse chromatin particles are divided into smaller angular pieces, and there is an increase in parachromatin The latter is due in part to an increase in the number of mitochondria and other cytoplasmic constituents The blister–supravital *technique confirms these findings* by *the* continuous direct observation of these changes on individual cells.

Phagocytosis by small lymphocytes per se has been documented Al though poor, this ability is genuine. The small amount of cytoplasm is one limiting factor It is of interest that in the area of inflammation, they often show an increase in phagocytosis that is greater than the proportional increase in cytoplasmic mass

Although this may be related to the various cytochemical changes, such as the increase in mitochondria, the specific relation to these alterations has not been clearly defined

The number of leukocytes in the peripheral blood will affect the quality and quantity of the inflammatory exudate In the patient with monocytic leukemia, as well as in animals with a monocytosis, if monocytes are present in sufficient number, they can represent the chief source of hemato genous macrophages The nuclear changes characteristic of monocytes, their increased size, precocious phagocytosis, etc , are evident during the early hours of the response Lymphopenia, without a concurrent monocytosis, will lead to a marked decrease in the number of lymphocytogenous macrophages Recently Page and Good[6] reported that in neutrophenic subjects the entire leukocytic cycle is interrupted Thus, the variations in circulating leukocytes can markedly influence the chain of events in the complex reaction to injury called inflammation

SUMMARY

The lymphocytes play a dynamic role in inflammation *They partake in* many interrelated functions In the defensive functions concerned with phagocytosis, the lymphocytes are an important source of motile macrophages The transformation of lymphocytes into macrophages in acute inflammation has been described and discussed

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albumin was observed by studying the elimination of antigen[54] and was measured by determining the amount of circulating antibody in the sera of recipients after the antigen was eliminated.[55]

Smears of the donor cells before transfer were stained using Papanicolaou and Wright's stains. The recipients were sacrificed at various times during the experiments, and tissue from the injection sites was fixed in Formalin



FIG 7-1 Preinjection smear, lymph node cells Wright's stain (× 1300)

for permanent sections In some experiments performed by Drs Neil and Dixon,[51] the tissue was quick frozen to provide material for immunohistochemical study of the sites for antibody, according to the Coon's technique,[56] and smears were made from loose cells in the transfer sites Both hematoxylin-eosin and pyronine-methyl green stains were used on the fixed material

Controls used in the cell transfer studies included (1) the transfer of cells to recipients with no subsequent injections of antigen—for detection of significant residual production of antibody by the cells and passive transfer of antibody, (2) the injection of antigen into x-rayed recipients which did not receive cells—for detection of lack of x-radiation effect on the ablation

of the immune response in the recipients; and (3) the transfer of cells killed by repeated freezing and thawing or by incubating at 60° C. until trypan blue stains showed dye uptake by over 75 per cent of the cells. Killed cells were transferred to (1) observe the host reaction to the transferred cells and

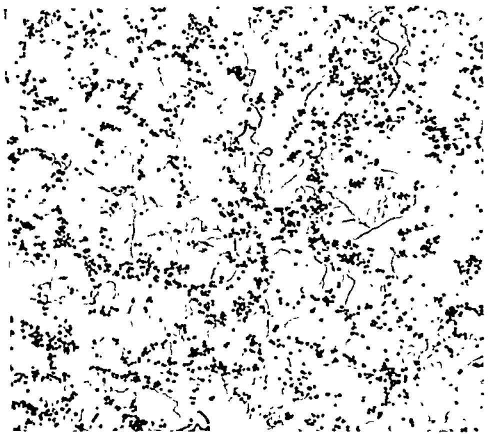


FIG. 7.2 Recipient site 1 day after transfer of living lymph node cells. Hematoxylin-eosin stain (× 300)

(2) determine if the production of anti bovine serum albumin needed viable cells.

RESULTS

The transferred donor cells were composed of approximately 90 per cent lymphocytes, 8 per cent macrophages and reticuloendothelial cells, and 2 per cent plasma cells (Fig. 7-1).

When live cells were transferred and subsequently stimulated with anti-



FIG. 7.3 Recipient site 4 days after transfer of living lymph node cells. Perivascular collection in muscle. Hematoxylin-eosin stain. (× 1300)

gen, the injection sites on the first day contained numerous readily visible, well-preserved lymphocytes, a few heterophils, and a few macrophages (Fig. 7-2); immunohistochemical stains for antibody were negative. Between days one and three the number of lymphocytes decreased, and occasional large, immature-appearing cells, resembling "Fagraeus' transitional cells"[14] or

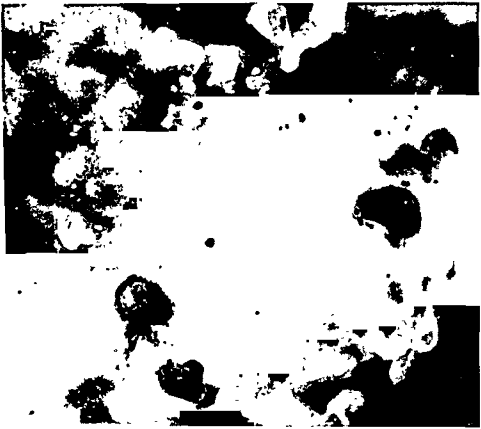


FIG 7-4 Frozen section of cell transfer on the third day, stained for anti BSA with use of Coons' fluorescent antibody technique The positive cells have white cytoplasm and are located around a medium sized blood vessel (× 800)

"Rich's acute splenic tumor cells,"[39] as well as smaller "preplasma" cells appeared (Fig 7-3) These cells tended to aggregate around nerves and blood vessels The first immunohistochemically demonstrated antibody was seen by the third day (Fig 7-4) Most of the antibody-containing cells were preplasma cells Some fluorescence was also present in the larger "transitional" cells Only a rare mature plasma cell was seen By the fifth day lymphocytes

were scarce, and almost as many plasma cells as preplasma cells were present. At this time fluorescent staining of antibody reached a peak in the sites (Fig 7-5). By the eighth or ninth days after transfer, virtually all the cells in the transfer sites were plasma cells, but fluorescent staining of antibody was waning (Figs 7-6 through 7-9)

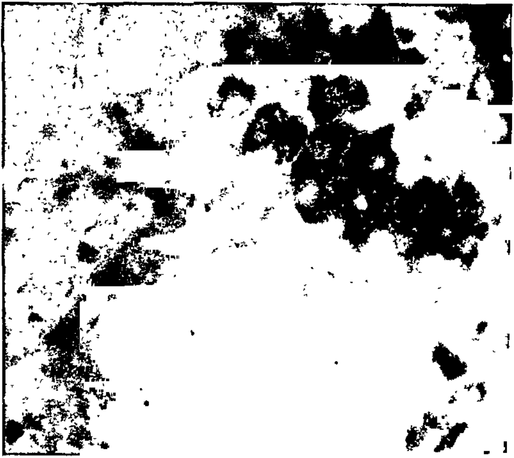


FIG 7 5 Frozen tissue section of cell transfer on the fifth day, stained for anti-BSA Note the increased number of positive cells around a medium-sized blood vessel as compared to the third day (Fig 7-4) (× 500)

Mitotic activity in the sites was minimal throughout the period of observation. Although an occasional binucleate cell was observed on tissue smears, no mitoses were found When mitotic counts were made on the fixed sites, no more than one mitosis per 500 cell nuclei was observed, and mitoses in the sites of killed cells were about as common (or uncommon) as in the sites of live cells

When dead cells were transferred, heterophils, host macrophages, and giant cells were present in the transfer sites, but there was an insignificant development of plasma cell precursors or plasma cells.

When live cells were transferred and not stimulated by antigen, the sites contained only rare plasma cells on the ninth day.

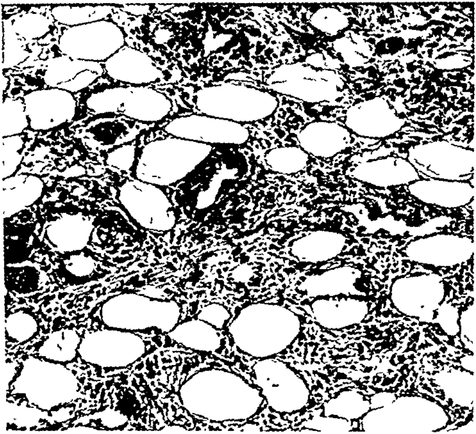


FIG. 7.6. Recipient site 8 days after transfer of living lymph node cells. Hematoxylin-eosin stain (× 140).

The immune responses in the experiments in which live cells were transferred and stimulated by antigen were of the secondary or hyperimmune type. On the basis of available functioning lymphoid cells, the responses in these recipients were comparable in quantity and quality of antibody to responses observed in intact hyperimmunized rabbits.[55, 60, 61] The elimination of antigen, a reflection of antibody production, was usually complete by the fifth or sixth day in the recipients, whereas rabbits undergoing a

primary response to bovine serum albumin do not eliminate antigen until the tenth or twelfth day after stimulation. None of the x-rayed recipients which received only antigen demonstrated an immune pattern of antigen elimination. No antibody was detected in the sera of those rabbits which

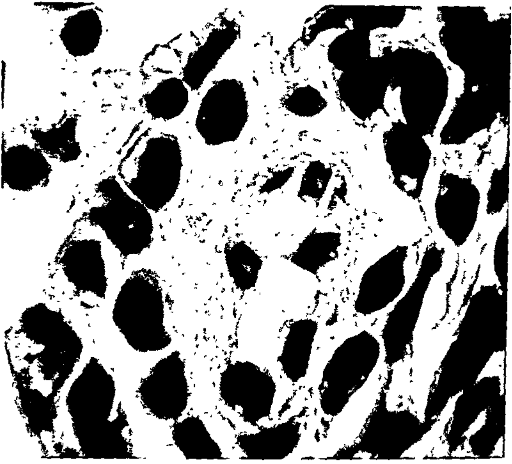


FIG 7 7 Recipient site 8 days after transfer of living lymph node cells Hematoxylin eosin stain (× 1300)

received only live cells without antigen, rabbits which received killed cells and antigen did not have an immune pattern of antigen elimination

The maximum concentration of circulating precipitating antibody which was present in the sera of recipients from three to four days after the elimination of antigen averaged about 20 μg. of antibody nitrogen in each milliliter of serum At this time the weight of the total antibody in the recipient was equivalent to approximately one fourth of the wet weight of the transferred lymphocytes When that value is converted to molecules of antibody per

transferred cell, somewhat more than 200 million molecules of antibody to bovine serum albumin were circulating in the recipients for each cell transferred eight or nine days before

DISCUSSION

On these observations we can base estimates concerning the minimum antibody synthesis resulting from the transfer of a known number of cells. First,

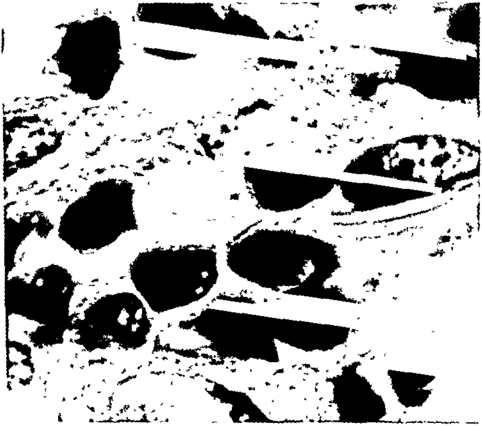


FIG 7-8 Recipient site 8 days after transfer of living lymph node cells Another area Hematoxylin-eosin stain (× 1800)

the total antibody synthesis during the first eight days of the response, most of which occurs from the third through the sixth day after injection, is probably two or more times that present in the recipient on the eighth or ninth day, because considerable antibody combines with circulating antigen and is rapidly catabolized[63] Additional antibody is lost also by normal non-

immune catabolism prior to the eighth day.[62] Next, not all the transferred cells have an opportunity to synthesize antibody since many die within the first few days after transfer. We cannot quantify the proportion of transferred cells that are lost, but we can minimize this variable by basing estimates of production on the recipients with the greatest amounts of circulating



FIG 7 9 Recipient site 8 days after transfer of living lymph node cells Pyronine methyl green stain shows intense cytoplasmic pyroninophilia of plasma cells (× 1300)

antibody These recipients had antibody levels of about 100 μg of antibody nitrogen per milliliter of serum, equivalent to one billion molecules of antibody per transferred cell Thus, in these instances the transferred lymph node cells were responsible for the synthesis of more than twice their own wet weight of antibody within eight days This places antibody-producing cells among the most active protein producing cells in the body

It seems likely that the transferred cells were responsible for the antibody

synthesis in the recipients because: (1) viability was a necessary criterion for the donor cells to produce antibody, i.e., no immune response was associated with the transfer of frozen thawed or heat-killed cells; (2) the recipient rabbits were exposed to 400 r whole body x-radiation which rendered them incapable of responding to bovine serum albumin; (3) the prompt elimination of antigen and the appearance of circulating antibody were typical of a secondary response, as would be expected from the hyperimmunized donor cells, (4) the quality of the antibody as measured by initial combining ratios was definitely that of secondary antibody; and (5) the amount of antibody observed in the recipients was much more than was observed in normal rabbits making a primary response to 1.5 mg. of bovine serum albumin.[63]

The quantity of antibody synthesized also enters into consideration of the question of which cell type was basically responsible for the antibody synthesis. Since the antibody made by the transferred cells in the recipient was comparable on a molecule per cell ratio to that made in the most active intact hyperimmunized rabbits, it seems most reasonable to assume that the cell responsible for the synthesis of antibody was the preponderant cell, the lymphocyte. To attribute the synthesis of so much antibody to the relatively few nonlymphocytes of the transferred pools seems unreasonable, since this would mean that the macrophages, reticuloendothelial cells, and plasma cells made many times their own wet weight of antibody in the eight-day period. A significant increase in the numbers of these cell types after transfer seems unlikely in view of the paucity of detectable mitoses in the cell transfer sites.

The failure to find mitoses in the transferred cells is in contrast to observations by Doctor Coons and his group who found considerable mitotic activity in the plasma cell series during the process of antibody formation in lymph nodes of the intact rabbit.[64] Similar to our cell transfer findings, less mitotic activity was noted by Fagraeus in cultured tissues producing antibody than in the same tissues of intact animals producing antibody.[14] Thus, while proliferation of antibody-containing cells during the course of an immune response may occur in the usual situation, it is apparently not an essential feature of the secondary antibody response made by transferred cells.

Since the immunologic evidence indicated that the transferred cells synthesized the antibody observed in the recipient of live cells, and since the decrease in lymphocytes observed between the second and fifth days after transfer was paralleled by an increase in antibody-containing cells of the transitional and preplasma types, and since S^{35} uptake studies on intact animals have shown that the peak rate of antibody synthesis occurred during approximately the same period, days 3 to 6 after the injection of antigen,[65]

it seems reasonable to conclude that the lymphocytes initiated the synthesis of antibody and that during this period of synthesis metamorphosed to transitional cells, then to preplasma cells, and finally to plasma cells (Table 7-2).

Table 7-2. Live Lymph Node Cells in Adult Recipients: Summary of Observations

Day after transfer	*Predominant cells*	*Antibody fluorescence*	*Antibody synthesis S^{35} intact rabbits*	*Circulating Antigen*	*Circulating Antibody*
1	Lymphocytes		+	++++	0
2	Lymphocytes	0	++	+++	0
3	Lymphocytes and preplasma cells	+	++++	+++	0
4	Preplasma cells	+++	++++	++	0
5	Preplasma cells	++++	++++	+ or ±	±
6	Plasma cells	+++	++	0	+
8	Plasma cells	++	+	0	+++
9	Plasma cells	+	+	0	++++

These experiments are not to be interpreted as denying the roles of other cells of the mesenchyme in the synthesis of precipitating antibody but rather to affirm the possibility that the lymphocyte can synthesize classic precipitating antibody. Similar experiments using oil-induced peritoneal macrophages obtained from hyperimmunized donor rabbits and transferred to x-radiated recipient rabbits have shown almost equal immunologic results.[48] Morphologically there was a decrease in macrophages paralleled by an increase in first transitional cells, then preplasma cells, and finally in adult plasma cells. The sites on the eighth day after the transfer of live peritoneal macrophages were indistinguishable from the sites obtained on the eighth day after the transfer of live lymphocytes.

SUMMARY

Donor rabbits were hyperimmunized to a bovine serum protein, albumin. Known populations of viable lymph node cells obtained from these donors at a time when the production of antibody was low were transferred to immunologically inert recipients. When these cells were stimulated by bovine serum albumin in the recipients, specific antibody to bovine serum albumin was subsequently detected in the recipients' sera. On the basis of correlated immunologic and morphologic observations, the following conclusions seem warranted.

1. The transferred cells were responsible for the synthesis of antibody.
2. The lymphocytes in the transferred cells were probably the basic cells responsible for the process.
3. The lymphocytes metamorphosed during the synthesis of antibody to plasma cells, with intermediate cell types prominent during the most active phase of antibody synthesis.

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CHAPTER 8

LYMPHOCYTIC RESPONSE TO TISSUE TRANSPLANTATION*

T. N. HARRIS AND SUSANNA HARRIS

It has long been recognized that tissues cannot be successfully transplanted from animals of one species to another (heterologous transplant) or even between individuals of the same species (homologous transplant), with the exception of some special cases of homologous transplantation such as those involving members of an inbred strain (isologous) or homozygous twins. In recent years there has been a great deal of interest and activity in relation to this remarkable phenomenon of tissue specificity. Although many problems are still unsolved, there has been a great increase in the extent of our understanding of the mechanisms involved in the rejection of transplanted tissue and even the development, by Billingham, Brent, and Medawar, of experimental means of induction of prolonged acceptance of homologous grafts (actively acquired tolerance).[1] The studies in this area in recent decades have led to the general recognition of immunologic mechanisms as being involved in the rejection of transplanted tissue, and in many of the studies to be reviewed below, the cellular responses to transplanted tissue have been examined from the point of view of the immunologic reaction or defense of the host.

STUDIES OF CELLULAR RESPONSE TO TISSUE TRANSPLANTATION

Cellular Response at the Site of Transplantation

In early studies the cellular reaction of the host tissues was studied as essentially a local phenomenon. In 1912 DaFano observed lymphocytic in-

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filtrations at sites of tumor grafts and concluded that these were associated with the resistance of the recipient's tissues to the graft.[2] Bashford *et al* had reported that mice could be rendered relatively immune to a cancer graft by the injection of homologous living tissue at least ten days before[3] Later, Murphy found that a sharp increase in the absolute lymphocyte count of the circulating blood followed the implantation of malignant tissue and showed correlation between the degree of lymphocytic reaction and the retardation of growth of implanted tumor or tissue. In these and others of a long series of studies on this subject, Murphy postulated an association of the lymphocytic reaction with an immune process In support of such an association, Murphy was able to present substantial evidence from his data.[4] This lymphocytic infiltration was found in the case of heterologous as well as homologous grafts, and where this cellular reaction did not occur, in the irradiated animal or in the brain or in the embryo, the heterologous graft grew with no evidence of host resistance In such instances, however, resistance to the graft could be reestablished by the introduction of adult lymphoid tissue, even, in the case of the chick embryo, when the adult-splenic tissue was introduced at some distance from the heterologous graft. Murphy was not, however, able to determine how the lymphocytic infiltration or the regional lymphatic hyperplasia exerted their effects on the implanted tissue. Loeb also described lymphocytic infiltrations about homografts and likewise considered that their presence was associated with the defense of the host tissue against the graft[5]

In the past fifteen years there has been a new sequence of studies of lymphocytic reaction at sites of tissue transplantation. In the period between that of the earlier studies described above and these more recent ones, attention had been drawn to the immunologic functions of the lymphatic system by the direct experimental evidence which began with the work of McMaster and his colleagues in 1935[6] Perhaps this development contributed to the more definitive association, by recent workers, of the infiltrating lymphocytes with formal immunologic mechanisms of defense of the host tissue

In the classic study of skin homografting by Medawar in 1944, he found that by the eighth day after placing of a homograft, shortly before the beginning of the overt processes of graft rejection, the graft was densely invaded by cells, largely lymphocytes with some monocytes, frequently in the form of halos around blood vessels The local lymphatic vessels were also hypertrophied. Subsequently these lymphocytes became pyknotic and then broke down altogether, and additional lymphocytes, released by rupture of the lymphatic vessels, showed similar changes By this time the breakdown of the graft was complete These changes were observed in the case of homografts but not of autografts[7] In this study by Medawar, the importance of classic

immunologic phenomena in the rejection of homografted tissue was clearly established, by the accelerated rejection of second-set homografts, in comparison to first homografts, and by the observation that an increase in the amount of tissue introduced in a primary graft can cause a greater degree of acceleration of rejection of a second one. Possible relations of the infiltrating lymphocytes to such immunologic processes were discussed in this paper. Similar observations on the host tissue reaction were reported in the study of Billingham, Krohn, and Medawar, who also found lymphocytes the most abundant member of the invading mass of leukocytes of rabbit skin homografts.[8]

Darcy also studied the cellular reaction to homografts in the rabbit, in this case homografts of the submaxillary gland. He found that the cells invading the graft included small lymphocytes and a large number of cells stained strongly with pyronine, identified as mature and immature plasma cells. The numbers of lymphocytes and of mature and immature plasma cells present in the graft at various stages after implantation were studied in the case of both first- and second-set grafts. Attempts to correlate the concentration of each of the cell types with the speed of graft destruction, the amount of grafted tissue destroyed, and the difference between reactions to first and second homografts led the author to conclude that the plasma cells that infiltrate the grafts are not a significant cause of their destruction. The same appeared to hold, although with less evidence, for the lymphocytes.[9]

In a study of human skin homografts, Rogers and colleagues observed the occurence of eosinophilic leukocytes in the blood and at the site of the homografts. They found some increase of eosinophils both in the blood and at the site of the graft, but this was of maximal intensity after the homograft had sloughed and only the dermis remained intact.[10]

In the case of tumor transplants, Toolan and Kidd inoculated mammary carcinoma and lymphosarcoma of C₃H strain mice into A mice. In tumors which regressed, they found a gradual shrinking of the tumor cells and increased basophilism of nuclear and cytoplasmic constituents, leading to complete breakdown of these cells. This sequence was found never to begin until "lymphoid elements" of the host accumulated and attached themselves to the peripheral cells, often curving like crescents around them as they shrank, while adjacent tumor cells without attached lymphocytes remained unaltered. In the case of mice in which such tumors had formerly grown and regressed, there was earlier arrival of lymphocytes, after second implantation, and correspondingly earlier regression of the tumor.[11]

Cellular Changes in Lymph Nodes Draining Sites of Homografts

The reaction of the lymphatic system to implanted tissue has also been studied by histologic examination of lymph nodes draining sites of implanta-

tion both of normal and malignant tissue. Gallone, Radici, and Riquire examined lymph nodes draining sites of skin homografting and found many large lymphoid cells with granules which stained with pyronine [12] In a more extensive study Scothorne and McGregor studied lymph nodes of rabbits regional to skin autografts and homografts in the ears of rabbits On the second day following homografting, the draining lymph nodes showed some enlargement of the medullary cords and some increase in mature plasma cells in these cords The major changes began on the fourth day, when accumulations of large lymphoid cells were found in the greatly enlarged cortex of the node, with similar changes of smaller degree in the medulla These cells contained pyroninophilic granules, but almost all this character of these cells was lost soon after graft destruction began. In the medulla the cords showed an increased number of mature plasma cells, and the sinuses contained many small lymphocytes as well as large lymphoid cells. These changes were not observed in lymph nodes contralateral to the homografted ear or in lymph nodes draining the site of autografts [13] In a subsequent study Scothorne treated skin-homografted rabbits with cortisone The graft was retained and was apparently healthy up to the end of the experimental period (16 days), and the large-lymphoid-cell response in the draining lymph nodes was entirely absent [14]

Lymph nodes draining sites of implantation of tumors have also been examined Ellis, Toolan, and Kidd implanted C_3H mouse mammary carcinomas and lymphosarcomas in a resistant strain of mice and then studied the regional lymph nodes [15] A few days after the implantation, the draining lymph node had become markedly hyperplastic, with an increase in the size and number of germinal centers and with thickening of medullary cords In the latter there was proliferation of elements that seemed to be young plasma cells

LYMPH NODE CELL TRANSFER IN RELATION TO THE IMMUNOLOGY OF TISSUE TRANSPLANTATION

In the early studies of tissue reactions at the site of homografts, various workers had concluded that the infiltrating cells were involved in a local defense of the host tissues against the transplant, although it was not known what the mechanism of this defense might be Later, histologic studies of lymph nodes regional to transplanted tissue had indicated substantial hyperplasia in these nodes of lymphoid cells—cells of the lymphocytic and plasmacytic series These changes were so similar to histologic observations which had been made in lymph nodes draining sites of injection of known antigens, such as those of bacterial, cellular, or viral origin, as to suggest that here, too, the lymphatic hyperplasia indicated a reaction to antigenic material in the transplanted tissue

It remained, however, for the work which will be described below to demonstrate experimentally that the cells of the regional lymph node were indeed capable of carrying out such immunologic reactions. The studies referred to are those involving the transfer of lymph node cells from an immunized or sensitized animal to a normal animal of the same species, a technique which has recently been used to a considerable extent in the elucidation of various immunologic phenomena. In the general pattern of these studies, lymph nodes or spleens have been removed from animals (donors) injected with antigenic materials. The lymph nodes or spleens have then been teased apart, and the cells thus released have been transferred to fresh homologous animals (recipients), which have then been examined for the appropriate immunologic reaction—hypersensitivity, humoral antibody, or resistance to transplanted tissue.

In studies involving a wide variety of antigenic substances, the data reported on cytologic examination of the suspensions of transferred cells have been fairly similar. In the reports of Chase[16] on suspensions of guinea pig lymph node cells, of Murchison[17] on analogous cells from mice, and of Roberts and Dixon[18] and of Harris and colleagues[19] on rabbit lymph node cells, the average percentage of cells of the lymphocytic series in the suspensions were $\geqq 95$, >95, 85, and 99, respectively. In connection with the cytologic composition of suspensions of transferred lymph node cells, it should be pointed out that Roberts, Dixon, and Weigle[20] and, more recently, Holub[21] have examined recipients' tissues at the sites of intramuscular and intraperitoneal injection of lymph node cells, respectively, and have found accumulations of plasma cells there, at about the same time as that of appearance of antibody in the serum. It was not possible in these studies to determine whether these plasma cells were the result of conversion of the transferred lymph node cells or of infiltration of host tissue cells. In the study by Roberts and colleagues,[22] there was a definite correlation between the presence of plasma cells and the appearance of antibody, but in the study by Holub, some plasma cell accumulation was found at the site of injection of lymph node cells even if these had not been incubated with antigen prior to transfer so that no antibody appeared in the serum of the recipient.

Lymph Node Cell Transfer Studies of Hypersensitivity and Antibody Formation

Before describing the studies that have been carried out with transferred lymph node cells in relation to tissue transplantation immunity, the immunologic potency of such cell suspensions will be indicated by a brief résumé of some of the recent literature on lymph node cell transfer in relation to hypersensitivity and to the production of classic serum antibodies.

The technique of lymph node cell transfer received its recent impetus

from the work of Chase, who succeeded in transferring hypersensitivity to tuberculin by cells from peritoneal exudates, lymph nodes, and spleens of tuberculin-sensitive guinea pigs.[22] In 1950 Chase reported the transfer to normal guinea pigs of skin hypersensitivity and anaphylactogenic antibodies to picryl chloride by injection of cells from peritoneal exudates, lymph nodes, and spleens of guinea pigs highly sensitive to picryl chloride. If, in such experiments, the donor animals had been injected with sheep erythrocytes, hemolysins could be found in the sera of the recipient animals. Extracts of the cell suspension, or cell suspensions which had been frozen and thawed, failed to produce the effect.[23] The transfer of tuberculin hypersensitivity has since been confirmed by many other workers.

In studies of humoral antibody production, Harris, Harris, and Farber injected dysentery bacilli into the feet of rabbits, excised the draining popliteal lymph nodes, and transferred suspensions of cells obtained from these nodes into fresh rabbits. Agglutinins to dysentery bacilli appeared in the sera of the recipients in a characteristic pattern.[24] Wager and Chase[25] and Stavitsky[26] reported the appearance of diphtheria antitoxin in recipients of cells obtained from spleens and lymph nodes of immunized donors. Roberts and Dixon transferred to irradiated recipients lymph node cells (popliteal, mesenteric, and axillary nodes) from donor rabbits immunized over a five-week period with bovine γ-globulin or bovine serum albumin. The recipients were then injected with radioiodinated bovine γ-globulin or with bovine serum albumin. The rate of elimination of these proteins from the circulation of the recipients was characteristic of a secondary response. The authors calculated that the total homologous antibody synthesized by the transferred cells during the first eight days of the secondary response amounted to approximately two thirds of the wet weight of the transferred cells.[19]

Recently, Sibal and Olson injected bovine serum albumin intravenously into hens and two days thereafter excised the spleens and obtained cell suspensions which were transplanted to the chorioallantoic membrane of embryonated eggs. The transplants were removed after from four to seven days of incubation, and extracts of these were found to contain low titers of antibody to bovine serum albumin, as indicated by adsorption-hemagglutination tests.[27] In experiments with guinea pigs, Rosenberg and collaborators[28] gave the donor animals a single intravenous injection of pooled human serum and hen egg albumin and removed the spleen and lymph nodes at various intervals thereafter. Cells obtained from these tissues were injected intradermally into recipient guinea pigs. Evidence of the development of antibody in the recipient was obtained by positive passive cutaneous anaphylaxis twenty-four hours or more after transfer.

In a number of studies, fragments of tissue have been transferred, rather than suspensions of cells In an early study (1930), Topley injected paratyphoid bacilli into rabbits intravenously. A day later the spleens of these rabbits were minced, and the fragments were injected intraperitoneally into fresh rabbits. The sera of the recipients developed agglutinins within a few days after transfer, which was earlier than would have been expected in the case of a primary response of the recipient to antigen present in the transplanted tissue[29] Fragraeus and Grabar transplanted fragments of splenic tissue from immunized donors into the peritoneum of recipients and subsequently found antibody in the sera of the latter.[30] Hale and Stoner obtained fragments of lymph nodes and spleen of mice which had received two injections of tetanus toxoid and transplanted these into the anterior chamber of the eyes of irradiated mice Antitoxin appeared in the sera of the recipients in low concentration.[31] Oakley, Warrack, and Batty injected diphtheria or tetanus toxoid into the interscapular fat of rabbits and between three and ten days after a secondary injection removed the fat and transplanted fragments of the tissue to the omentum of normal rabbits. Antitoxin appeared in the sera of the recipients subsequently[32]

In many of the studies referred to above, suspensions of cells or tissue fragments were injured by heating, freezing, and thawing or by treatment with distilled water before being transferred In all these the transfer of such preparations did not lead to the appearance of antibody in the corresponding recipient animal Transfer of cells or fragments to animals of other species has also been found to be ineffective (with the exception of a study reported by Wesslen.[34]

More recently it has been found possible in the case of some antigens to provide the contact between antigen and lymph node cells in vitro In experiments with lymph node cells from uninjected donors, Harris, Harris, and Farber found that such cells could be incubated in vitro with *Shigella paradysenteriae*, washed and transferred to irradiated recipients with the subsequent appearance of agglutinins to *Shigella* in the sera of the latter The appearance of antibody in the sera of recipients of such lymph node cells was later than in the case of lymph node cells which had been obtained from antigen-injected donor animals[18] In a later study a soluble form of the antigen was used for in vitro incubation with the lymph nodes cell, with results similar to those described[19]

In another study involving in vitro contact between antigen and, in this case, spleen cells, Sterzl reported the appearance of agglutinins following the intraperitoneal injection of five-day-old rabbits with spleen cells which had been incubated in vitro with *Salmonella paratyphi B*[35] Finally, Holub incubated cells from lymph (cisterna chyli) of rabbits in vitro with *Brucella*

suis and *S paratyphi*, transferred these to two- to five-day-old rabbits, and found homologous serum antibodies in 90 per cent of the recipients a few days after cell transfer.[21] In experiments involving bovine γ-globulin or bovine serum albumin as the antigen for in vitro incubation with lymph node cells, Roberts and Dixon were not able to detect antibody in the sera of recipient rabbits unless the recipients were also injected with the antigen [18]

LYMPH NODE CELL TRANSFER IN STUDIES OF TISSUE TRANSPLANTATION IMMUNITY

The strong body of evidence on the immunologic potency of lymph node cells has been applied by several workers in the field of tissue transplantation In studies of immunity to transplantable tumors, Mitchison[36, 37] obtained cells from mouse lymph nodes regional to solid tumor homografts and transferred them, either minced or in suspension, to a secondary host. It was found that when the recipient mouse was challenged by a homograft of the same tumor, it destroyed it two or three times more quickly than would otherwise have been the case. The transferred cells had the capacity to confer immunity on the recipient if the nodes were removed from the donor mouse at the time the grafted tumor was undergoing breakdown. Cells from non-regional lymph nodes and from the spleen, as well as serum or whole blood, failed to transfer immunity. Evidence was presented favoring the hypothesis that "the lymph node cells were immunologically activated before transfer, and that they conferred immunity by continuing to function in their host."[17] Homografts of a transplantable sarcoma gave rise to the production of serum antibody which could be detected by its cytotoxic action on the cells of the tumors and also by means of a hemagglutinin test Following the transfer of regional lymph node cells from mice with such grafts into hosts of the same strain, hemagglutinins could be detected in the host serum However, the capacity of the cells to transfer hemagglutinin production developed later than the power to transfer increased resistance to grafts Splenic cells also transferred hemagglutinin production, although to a lesser extent The conclusion was drawn that the hemagglutinating antibody is distinct from the antibody effective in protection against homografts [38]

In the area of skin homografting, Billingham, Brent, and Medawar gave mice of one strain skin homografts or injections of leukocytes from mice of a second strain and then transferred fragments or cells of the regional lymph nodes to other mice of the second strain by the intraperitoneal route The mice that received the lymph node tissue fragments or cells showed heightened resistance to further skin homografts from the strain used for immunization The transfer was not effective when contralateral lymph nodes were

used, or when tissue fragments were injected subcutaneously, or when tissue fragments had been frozen and then thawed or dried.[39]

In another approach to the demonstration of the association of lymph node cells with the transplantation immunity of skin homografts, Voisin and Maurer obtained cells from lymph nodes and peritoneal exudates (largely macrophages) of guinea pigs that had received skin homografts eighteen days previously. These cells were transferred to some of a group of guinea pigs that had received skin homografts three days earlier, from the same animals which had been the donors of the earlier skin graft. The other skin-grafted guinea pigs served as controls. On comparing the appearance of the skin grafts in these two groups between the seventh and eleventh days, it was found that the grafts of two thirds of the recipients injected with lymph node or peritoneal exudate cells were in worse condition than were the controls.[40]

In yet another approach to the role of cells of lymph nodes regional to the site of a skin homograft, Brent has described a series of experiments in guinea pigs. In these experiments skin was grafted from donor guinea pigs to recipients. Subsequently cells of the lymph nodes regional to the site of the graft were obtained and were injected intradermally into the donor guinea pigs. These injections were found to produce local inflammatory wheals, indicating that a *specific reaction had occurred* between the lymph node cells and the tissue antigens of the donor.[41]

Finally, lymph node cell transfer of tissue transplantation immunity has been studied in connection with the homotransfer of lymph node cells themselves. In the work with transfer of lymph node cells incubated in vitro with soluble material derived from *Shigella paradysenteriae*, referred to above, it was found that the usual appearance of agglutinins to *Shigella* after the transfer of such cells could be prevented by prior injection of the recipients with pooled leukocytes from donor animals. In order to bring about this suppressive effect on the lymph node cells, it was necessary to inject an adequate number of leukocytes at an appropriate interval prior to the lymph node cell transfer. These and other considerations suggested an immunologic mechanism of this suppressive effect, analogous to the accelerated rejection of a second skin graft of a given donor to a given recipient.[42-45] In these terms the recipient animal was regarded as having been actively immunized to the tissue transplantation antigens of the donor animals by the prior injection of the leukocytes. It was of interest to see whether in this situation the tissue transplantation immunity—i.e., the suppressive effect on transferred lymph node cells—could be transferred by appropriate lymph node cells. Accordingly, rabbits were injected in one hind foot pad with blood leukocytes pooled from a set of prospective lymph node cell

donors Four days later the ipsilateral popliteal lymph nodes were obtained from the leukocyte-injected rabbits, and cells obtained from these were transferred to the prospective recipients of the experiment. At the same time lymph node cells were obtained from the original donors of the blood leukocytes The latter were incubated in vitro with *Shigella* antigen, washed, and transferred to the recipient animals. On subsequent determination of the antibody titers of the recipient rabbits, it was found that those that had received the cells from lymph nodes draining sites of injection of rabbit leukocytes showed mean anti-*Shigella* agglutinin titers which were only approximately one seventh those of the control animals, which had been given only the *Shigella*-incubated lymph node cells Other control groups of recipients showed anti-*Shigella* agglutinin titers in the usual elevated range These included recipients in which the "anti-leukocyte" lymph node cells had been heated before transfer and included still others given cells from lymph nodes contralateral to the sites of rabbit-leukocyte injection.[43]

SUMMARY

It can be seen that the involvement of cells of the lymphocytic series, or of the lymphatic system, in the host response to transplanted tissue has been indicated by observations made in three types of studies. (1) studies of cellular infiltrations of transplanted tissue, (2) examinations of the cellular reactions in lymph nodes regional to tissue transplants, and (3) studies of the transfer of transplantation immunity by lymph node cells The precise function of the various cell types within this system in the rejection of transplanted tissue is not understood, and many questions remain unanswered in this area For example, is the presence of lymphocytes at the site of a homograft, and especially in contiguity with transplanted cells, an expression of hypersensitivity, or a means of delivering antibodies, or do these cells serve some other function in this situation? Again, in suspensions of transferred lymph node cells, which of the cell types are effective in conferring transplantation immunity? If the majority cell type found in the transferred suspensions is involved, does it carry out its function in the form in which it is transferred or after conversion to another cell type in the new host tissue, and by what mechanism is the tissue transplantation immunity conferred? The answers to some of the questions in this area will come from the considerable efforts being expended at present on immunologic study of these problems However, a great contribution to this field will be made by the clarification of the interrelations among cell types within the lymphatic system—a clarification that must result from studies by cytologists and pathologists

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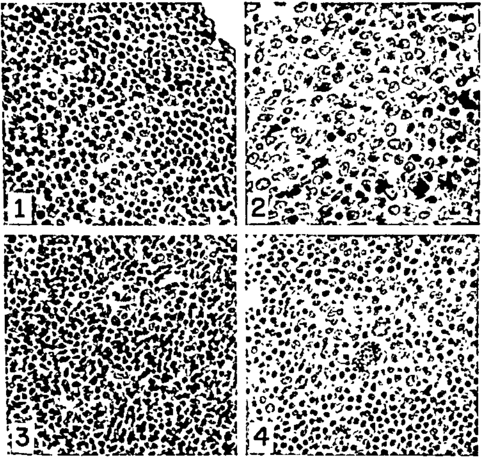


FIG 9-1 Inguinal lymph node of untreated control CBA mouse Note variation in size of lymphocytes in addition to cytoplasmic variations Hematoxylin eosin stain (× 425)

FIG 9 2. Lymph node of adrenalectomized CBA mouse Note increase in lymphocyte mean cell diameter and mitotic figures Hematoxylin-eosin stain. (× 425)

FIG 9 3 Treatment of intact CBA mouse with ACTH (5 days) Inguinal node showing pyknosis of lymphocytes Hematoxylin eosin stain (× 425)

FIG 9-4 Treatment of mouse with single large dose of adrenal cortical extract (I p) Note phagocytosis of broken small lymphocytes by macrophages Hematoxylin-eosin stain (× 425)

to be due to increased adrenocortical discharge due to the stress of the operation itself [5] Cytorrhexis does not last more than an hour or two, and then the lymphatic tissue undergoes a change toward that characteristically found in adrenalectomized animals [5]

The cytologic characteristics of lymph nodes of adrenalectomized animals are several There is less discrepancy in size between large lymphocytes and

CHAPTER 9

LYMPHOCYTOKARYORRHECTIC EFFECTS OF ADRENOCORTICAL STEROIDS

THOMAS F. DOUGHERTY

Several papers have been published which review the literature pertaining to the influence of steroid and other hormones on quantitative changes in lymphatic tissue mass, regulation of blood lymphocytes,[2] and the influence of stress and biochemical relationships of hormonal influence on lymphatic tissue[3] Very few papers have dealt with the effects of lymphocytokaryorrhectic hormones on the lymphocytes themselves The cellular response, of course, accounts for the changes in mass of this tissue and, ultimately, for the regulatory effects of steroid and other hormones on its growth and involution It has been only within the last few years that we have begun to understand some of the important features of the influence of adrenocortical hormones on the growth and maturation of lymphatic tissue

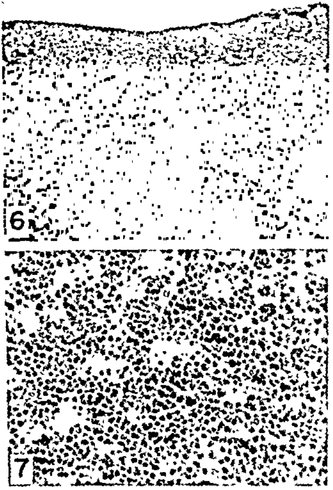
The physiologic importance of these events is, of course, enormous with respect to the formation of serum protein and the functioning of lymphatic tissue as a protein store and a most likely candidate for the source of protein for gluconeogenesis,[4] which is controlled by steroid hormones having essentially the same structure as those which influence lymphatic tissue Several of these features will be discussed at greater length below

MORPHOLOGIC CHANGES IN LYMPHATIC ORGANS FOLLOWING ADRENALECTOMY

Immediately after adrenalectomy of animals (mice and rats), pycnosis of lymphocytes with phagocytosis of nuclear debris frequently occurs in lymph nodes, the spleen, and other lymphatic organs This effect has been shown

water.[8] However, the nitrogen content of the whole lymphatic node increased as a reflection of the increased cellularity.[7]

The thymus also undergoes an increase in cellularity, and as a consequence the demarcation between cortex and medulla grows less distinct.



FIGS 9 6 and 9 7 Thymus of rabbit showing pitting effect of thymus following 10 mg ACTH (i p) Pitted areas are occupied by macrophages ingesting broken lymphocytes Hematoxylin-eosin stain (Fig 9 6 × 110, Fig 9 7, × 475)

The increase is in the thymic cortex, which is accompanied by a big increase in the number of lymphocytic mitoses The nitrogen content per milligram of thymus is not increased [7] The mean mass of the thymus increases as compared to nonadrenalectomized controls, and the nitrogen content reflects

small lymphocytes (Figs. 9-1, 9-2). Tiny pycnotic cells are absent, and there is an increase of cell size to that of a medium-sized, mature lymphocyte with a wide rim of clear cytoplasm. The cells do not appear to be more immature in that they do not acquire an increased nucleolar number or a more leptochromatic fineness of the chromatin pattern.[6] The cytoplasm increases in amount and appears very hyaline with a loss of cytoplasmic basophilia.

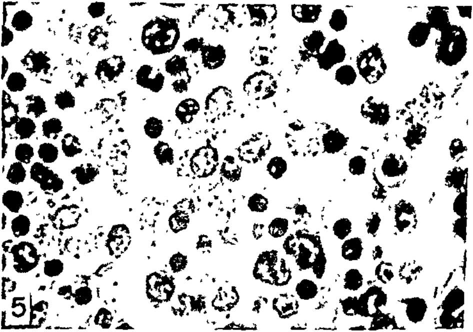
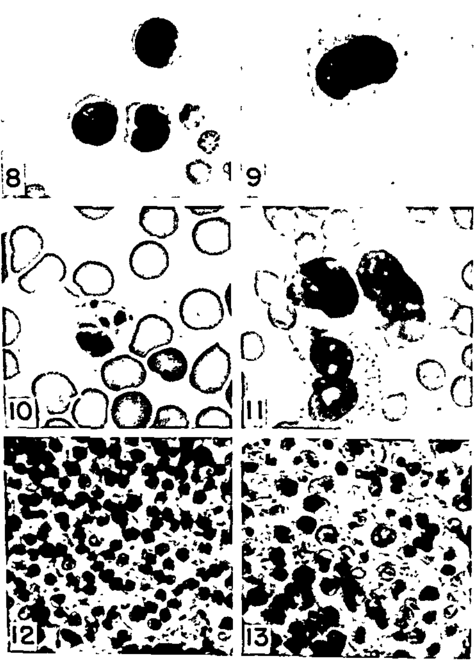


FIG. 9-5. Phagocytosis of nuclear and cytoplasmic debris by fixed reticuloendothelial cells in medulla of inguinal node following ACTH treatment. Hematoxylin eosin stain. (× 1350)

The cells are closely packed in the node (Figs. 9-1, 9-2). There is also a general tendency to have indefinite germinal centers in that there is no sharp demarcation of the difference between the large cells in the germinal center and the collar of lymphocytes about it.[6] Numerous mitoses are found, and studies of DNA renewal in this laboratory support the observation that there is a true increase in rate of proliferation as compared to nonoperated controls.[2]

The nitrogen content per unit weight of lymphatic organs of adrenalectomized mice does not increase.[7] Therefore it is apparent that the larger lymphocytes in adrenalectomized animals do not contain more protein per cell. This increase in cell size is probably due to increased intracellular


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this change in mass.[7] Similar but less striking changes are observed in other lymphatic organs (Peyer's patches, etc.).

The blood lymphocytes of adrenalectomized animals tend to undergo changes similar to those found in the lymph nodes[8] In stressed adrenalectomized animals this increase in cellular size (Fig. 9-11) and change in staining capacity of cytoplasm of blood lymphocytes occurs more rapidly.[8] Immediately following adrenalectomy, there is a decrease in the number of blood lymphocytes, followed by reconstitution to normal levels at about one hour, and by four hours in the adrenalectomized mouse, the characteristic cellular changes appear.[9] There is an increase in the size of the nucleus as well as a marked increase in the amount of the cytoplasm[8] which has a hyaline appearance (Figs 9-8, 9-11). It is apparent from numerous studies (both experimental and clinical) that mean blood lymphocyte size reflects the amount of cortisol in the blood which is acting on the cells.[10, 11] These cells, which have been called "stress" cells,[8] are characteristically found in adrenalectomized animals and in cases of human adrenal insufficiency.[11] Stress lymphocytes have been shown to be more resistant to the cytorrhectic effect of cortisol than the lymphocytes of similar mean diameter in a state of *eucorticism*.[10]

THE EFFECTS OF ADRENAL CORTICAL HORMONES ON LYMPHOCYTES

Reduction of lymphatic tissue weight by adrenocortical hormones is accomplished by a destruction of lymphocytes (lymphocytokaryorrhexis) by inhibiting the mitosis of lymphocytes, and under conditions of prolonged hyperadrenocortical hormone concentration, cortisol inhibits differentiation from immature cells (heteroplasia)[8]

The lymphocytokaryorrhexis begins by a shedding or budding off of cytoplasm (Fig 9-9). This is most marked in medium-sized mature lymphocytes in *both the lymphatic organs* and in *the blood*[8, 12] *This shedding of* cytoplasm is remarkable and has been photographed by time-lapse cinematography in our laboratory[13] The buds come off by cytoplasmic rounding

FIG 9 8 Normal lymphocytes of CBA mouse May Grunwald Giemsa stain (× 950)

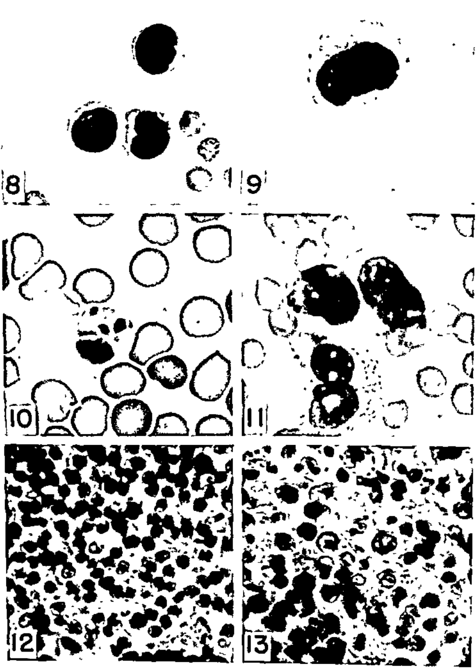
FIG 9 9 Budding lymphocyte of CBA mouse following cortisol treatment (0 1 mg i p) May Grunwald Giemsa stain (× 950)

FIG 9 10 Lymphocyte of CBA mouse following large cortisol dose (1 mg, i p) May-Grunwald-Giemsa stain (× 1500)

FIG 9 11 Lymphocytes and eosinophile of adrenalectomized CBA mouse Note marked increase in amount of lymphocytic cytoplasm May Grunwald Giemsa stain (× 950)

FIG. 9 12. Chronic cortisol treatment of CBA mouse (1 mg /day for 9 days) Note uniformity of pyknotic lymphocytes Hematoxylin eosin stain (× 650)

FIG 9 13 Same as Fig 9 12 24 hours after last injection Note increase in cytoplasm of small cells and large number of *reticular lymphocytes Hematoxylin-eosin stain* (× 650)


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up (Fig. 9-9) and for a time remain attached to the rest of the cytoplasm by a very thin filament which eventually breaks and the bud floats away. When this process is going on rapidly, one finds the efferent lymph loaded with pieces of cytoplasm bearing a superficial resemblance to platelets. These pieces of cytoplasm were described by Downey and Weidenreich,[14] who called them hyaline bodies Although these authors did not relate them to a hormone-mediated effect, they did show that this effect was enhanced in animals treated with cinnabar, and they also postulated that it was a secretory phenomenon by which constituents of the cell cytoplasm were delivered to the lymph.

Following shedding of cytoplasm, and appearing as though it were an extension of this effect, the nucleus begins to shrink in size, loses its characteristic chromatin pattern, becomes irregular, pycnotic, and hyperchromatic, and subsequently may break into pieces (karyorrhexis).[6] Some years ago, when this effect was first described in the endocrinologic literature, it was called lymphocytolysis. Actually, this destructive effect is not a lytic but a karyorrhectic phenomenon. We are certain that the cytoplasmic loss is not necessarily total but may be a partial loss [15] Further, cortisol does not necessarily produce nuclear destruction of all lymphocytes [15] A given quantity of cortisol acting upon a variety of lymphocytes of different sizes and having different amounts of cytoplasm appears to produce graded effects on the cells, depending upon the maturity and size of the lymphocytes The most immature cells, such as reticular lymphocytes, are least affected by cortisol (Figs 9-3, 9-5) [6] Conversely, small lymphocytes are extremely susceptible to the lymphocytorrhectic effects of this hormone [16] Medium-sized lymphocytes appear to have intermediate susceptibility to hormone action. These cells, however, display the most dramatic budding effects Malignant lymphocytes are similar to reticular lymphocytes in their resistance to both the budding and lymphocytorrhectic hormonal effects [17]

Subsequent to cellular destruction, there is phagocytosis of both nuclear and cytoplasmic material (Fig 9-5) [6] Within a few hours following cortisol administration, there is very little remaining evidence of cellular destruction [6] The rates of destruction, phagocytosis of nuclear and cytoplasmic material, and intracellular digestion are extremely rapid, so that unless tissues are observed at various stages of this cyclic process, these events may not be seen It should be emphasized at this point that the cortisol effects are due to direct action of the hormone and occur *in vitro* as well as *in vivo*.[13] Extensive studies have been performed in our laboratory on the structure activity relationships of various closely related steroid hormones which regulate lymphatic cell growth and destruction [16] The details of these observations may be found elsewhere as may similar studies performed using malignant lymphocytes [16]

During the period of acute hormone action, there is an inhibition of mitosis which lasts for several hours and is followed by a resumption of cell division until there is a reconstitution of lymphatic organs characteristic of the age and sex of the animal.[6] This effect of lymphocytokaryorrhexis, dissolution of cells and inhibition of mitosis and growth of lymphocytes, lasts for only a short time following a single increase in the concentration of lymphocytokaryorrhectic hormones at the cell site.[2]

CHRONIC EFFECTS OF CORTISOL ON LYMPHATIC TISSUE

Persistent cortisol effects can be produced only when animals are treated at closely spaced intervals with either ACTH or appropriate steroid hormones.[18] Similarly, constant stress must be maintained to establish prolonged reduction of lymphatic tissue.[18] During the period of chronic cortisol treatment, very little lymphocytorrhexis is observed. The lymphocytes are pycnotic and hyperchromatic and have scant cytoplasm (Fig. 9-12). No evidence of homoplastic or heteroplastic growth of lymphocytes is seen in chronically treated animals. However, following either acute or chronic treatment of lymphatic tissue with lymphocytokaryorrhectic hormones, both the reticuloendothelial cells and the most immature reticular lymphocytes appear to be highly resistant to the destructive effects of these hormones (Fig. 9-12).[6, 15] Thus, since progenitive cells resist lymphocytokaryorrhectic action, a seed bed of germinative cells is retained for repopulation.

REPOPULATION OF LYMPHATIC TISSUE

Reconstitution of lymphatic tissue has been investigated following prolonged treatment with cortisol and subsequent hormone withdrawal and following hormone treatment and then adrenalectomy.[18] At the termination of the experiments, lymphatic organs were removed, weighed, and sectioned for histologic study. The lymph nodes become more rapidly reconstituted than the thymus which is very slow in the rate of its return following cortisol- or ACTH-induced involution.[18] This is also true following various stressful stimuli. Of most interest here is the fact that within hours following cessation of hormone treatment, there appears to be an increase in mean cell size in the lymph nodes (Fig. 9-13). At this time one sees little evidence of mitosis and heteroplasia. Rather, it appears that lymphocytes may become and remain pycnotic for some time and return to their previous states when the hormone level diminishes.[15] Reconstitution, therefore, is accomplished in part from these surviving cells.

DIFFERENTIAL SUSCEPTIBILITY OF LYMPHOCYTES TO HORMONE ACTION

We have postulated for some years that maturation of immature lymphocytes occurs in part as a consequence of the continuous action of cortisol on these cells. The lymphocytic cytoplasm is reduced gradually, and eventually the nucleus decreases in a fashion similar to that found in the erythroblastic series. Finally, there is a complete loss of cytoplasm and nuclear integrity This gradual process is enhanced when larger than normal amounts of hormone are available to act on the maturing lymphocytes. The overwhelming amount of evidence indicates that the effects of cortisol described above on lymphatic tissue are constant and physiologic The results of hyperconcentration appear to be exaggerated normal responses

REUTILIZATION OF NUCLEAR AND CYTOPLASMIC MATERIAL

When the histologic effects of adrenocortical hormones on lymphatic tissue were first described, attention was called to the fact that changes occurred in the reticuloendothelial cells as well as in lymphocytes.[6] The point was made at that time that when lymphocytes are destroyed, they are subsequently phagocytized by both fixed and free histiocytes (Figs 9-4, 9-6, 9-7)[6] The fixed reticular stroma cells phagocytized both nuclear and cytoplasmic particles (Fig 9-5) It was apparent, too, that the lymphocytes become highly basophilic and resemble certain types of plasma cells[6, 19] The suggestion was made that there was an ingestion and retention of reticular cells of destroyed lymphocytes and that nuclear and cytoplasmic material could be reutilized in formation of new cellular constituents during heteroplastic growth This subject has been discussed in detail recently by White[20]

The possibility of reutilization of nucleic acid constituents and even nonmetabolized DNA and RNA by lymphocytes has recently been emphasized[21, 22] It appears to be conclusive that at least a portion of nucleoprotein from destroyed lymphocytes may be reincorporated in immature cells

The significance of this finding is enhanced in view of suggestions made by numerous authors that lymphocytes are derived by heteroplasia from reticuloendothelial cells Therefore, the template for protein synthesis (particularly antibody protein) may be retained in the system of cells which synthesize antibody This concept is in line with our theory that antibody synthesis starts in the most immature lymphocytes and continues to progress as these cells differentiate[15] Thus, when adrenocortical hormones destroy mature lymphocytes, they release already formed antibody and also make possible the phagocytosis of nuclear and cytoplasmic material by reticuloendothelial cells, and thus the process of differentiation may be renewed

STRUCTURE ACTIVITY RELATIONSHIPS

There are marked differences in lymphocytokaryorrhectic capacity of the various adrenal cortical hormones Cortisol, which is the naturally secreted adrenal hormone of the human, is the most potent. Other less effective hormones are cortisone, corticosterone, and dehydrocorticosterone or compound A.[16] None of the other adrenal cortical secretory products appear to have this capacity. Analysis of both the naturally occurring hormones and their analogs which are lacking in one or more substitutions on the gonane nucleus indicates that an unsaturation of the A-ring, a ketone at position 3, either an oxy or hydroxyl substitution at position 11, and a side chain with an alcohol at the 21 position are necessary. Two compounds both lacking a 17-hydroxy grouping are corticosterone and 11-dehydrocorticosterone.[16] Both of these compounds are effective in suppressing lymphatic tissue growth and bringing about lymphocytokaryorrhexis It is interesting in this respect that these compounds have little or no anti-inflammatory activity, and thus there is a separation of anti-inflammatory and lymphocytorrhectic effects

MECHANISMS OF HORMONE ACTION

The effects of cortisol are direct, i e , the lymphocytokaryorrhectic activity of this hormone is manifest in vitro and in vivo Further, these hormones do not act in an all-or-none fashion but in a dose response manner. Therefore, the greater the dose, the greater the degree of lymphocytokaryorrhexis However, it is interesting that cortisol among the effective hormones has a greater involutionary action on the lymph nodes than any of the hormones The explanation offered for cortisol activity is that it has a greater capacity to destroy less-matured lymphocytes than its analogs Thus, there is a qualitative as well as a quantitative difference in the various adrenocortical steroids which appears to be determined by the degree of maturity of lymphocytes available for hormone action The duration of cortisol action on lymphocytes is short. Radioactive hormone (free 1-C^{14} cortisol) does not remain in lymphatic tissue more than five or six minutes following intravenous administration However, the major effects of cortisol on lymphocytes persist for much longer periods of time than cortisol, or its metabolic products are present in lymph nodes, spleens, and thymi[23] This indicates that cortisol triggers a process in the cell which is self perpetuating The lack of necessity for continuously increased hormone concentration at the site of anti-inflammatory or gluconeogenic activity[24] is similar to that found for effects on lymphocytes[23]

It is evident, then, that one way that leukemic lymphocytes can maintain their integrity and continue to multiply is by their capacity to inactivate cortisol, the normally occurring maturing hormone for the lymphocyte It

is tempting to speculate, and indeed seems warranted, that once an alteration has occurred in such a way that the immature cell can continue to resist maturing effects of cortisol, the seed bed may continue to grow and that this possibly may be essentially the situation in lymphatic leukemia.

As discussed above, the greatest effect of cortisol on lymphocytes is on the small cells, which in turn, we now know, are incapable of bringing about any demonstrable catabolic changes in this hormone.[25] Immature lymphocytes and lymphatic leukemic cells have been shown to have the capacity to convert cortisol to several steroids which lack any observable lymphocytorrhectic effect.

SUMMARY

It is apparent that cortisol acts as such and not through a metabolically altered form of this molecule. Before we speculate on its mechanism of action on lymphocytes, several points should be re-emphasized. One is that immature lymphocytes and leukemic cells can catabolize cortisol. They convert it into several products which do not possess any lymphocytokaryorrhectic activity. This, of course, emphasizes the fact that the malignant cell is able to maintain its integrity and activity because it can inactivate a hormone which destroys its normal counterpart. The process of budding and lymphocytorrhexis once initiated by cortisol continues when this hormone is either gone or has been converted to other noneffective steroids With these points in mind, one may speculate, then, as to why cortisol produces its effect on the lymphocyte The possibilities by which the hormonal lymphocytorrhectic effect occurs could be threefold·

1. *Enzymic* the effect could be through loss of enzymatic activity on the catabolism of the hormone by the most mature cells and/or a loss of the coenzymes necessary for the transhydrogenation of the steroids (DPN and TPNH) which are essential for the inactivation of the hormone[26]
2. *Transport* the effect could be directly on the cell membrane, and the permeability of the hormone to get at the enzyme-coenzyme system is reduced, therefore, the inactivation would also be decreased
3. A combination of these two possibilities

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CHAPTER 10

RADIATION EFFECTS ON LYMPHOCYTES

ROBERT SCHREK

The lymphocyte is a unique cell It is the only nonmitotic cell that is sensitive to small doses of x-rays In contrast, the granulocyte is highly radioresistant. In this paper I shall consider not only the biologic reactions of lymphoid tissue to x-rays but also the use of x-rays as a tool to study the physiology of the lymphocyte I shall first review the in vitro findings as these have clarified the in vivo observations

IN VITRO STUDIES

The morphologic effects of x-rays on lymphocytes in vitro have been studied by time-lapse cinemicrography [49] Three hours after in vitro irradiation of rabbit lymphocytes with 1000 r, most of the cells still appear morphologically normal (Fig 10-1) The photomicrographs were taken with phase contrast microscopy and show clearly the nucleus and cytoplasm, the nuclear wall, and the chromatin masses in the living unstained lymphocyte The first degenerative change in the irradiated cell is the appearance of a small intranuclear vacuole (Fig 10-2) Shortly after the onset of the vacuole, the cell and the nucleus usually start to change shape rapidly with the formation of lobulations (Fig 10-3) Frequently each lobule has a separate fragment of nucleus as is seen in cell F in Figure 10-3 and still more clearly in Figure 10-4 where the cell seems divided into two separate parts, each with a vacuolated nucleus The vacuoles continue to grow, pushing the chromatin peripherally to form a ring or a sphere of chromatin In about 20 minutes after onset, the vacuole ruptures the chromatin ring as is seen in cell C, Figure 10-5, and the chromatin appears horseshoe-shaped The degenerated chromatin material is apparently fluid and contracts to form a crescent, a

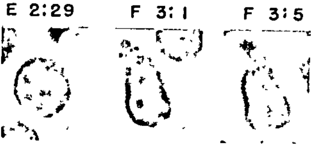


FIG 10-1. Morphologically normal lymphocytes *Note:* Figs. 10-1 through 10-6 are prints from a 16 mm cinemicrographic film of rabbit lymphocytes irradiated with 1000 r. The headings give a code letter for the individual cell and the time of incubation at 37° C after irradiation in hours and minutes (× 1750)



FIG 10-2 Early intranuclear vacuoles in lymphocytes irradiated with 1000 r (See Note, Fig 10 1)

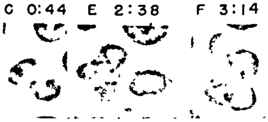


FIG 10-3 Early changes in shape of irradiated lymphocytes and their nuclei (See Note, Fig 10-1)

semicircle, and finally a small pyknotic and sometimes fragmented nucleus (Fig. 10-6) The nonirradiated lymphocyte also dies by the process of intranuclear vacuolization (Fig 10-7) The only difference is that the nonirradiated cell develops intranuclear vacuoles later than the irradiated cell. It seems that irradiation with 1000 r or less does not produce any new reactions in the lymphocyte but only accelerates normal degenerative changes

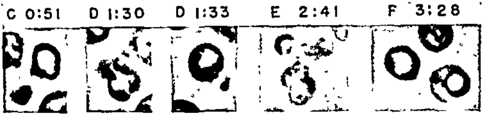


Fig 10-4 Well developed intranuclear vacuoles associated with lobulation of irradiated lymphocytes (See Note, Fig 10-1.)

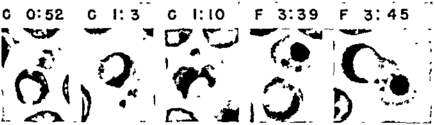


Fig 10-5 Rupture of chromatin ring and early contraction of chromatin material in irradiated lymphocytes (See Note, Fig 10-1)

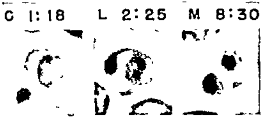


Fig 10-6 Pyknosis and fragmentation of nuclei in irradiated lymphocytes (See Note, Fig 10-1)

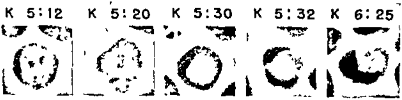


Fig 10-7 Changes in a nonirradiated rabbit lymphocyte Prints made from a cinemicrographic film (× 1750)

After irradiation with 2000 r or more, some rabbit lymphocytes develop nuclear abnormalities such as bilobed and trilobed nuclei.[50, 51] About one to two hours after irradiation, both the lymphocytes with normal nuclei and those with lobed nuclei undergo sudden acute death with only slight changes in morphology. I have called this change "death by delayed fixation." On further incubation, the "fixed" lymphocytes undergo post-mortem autolytic changes characterized by slow shrinkage of the nuclei and blurring of the chromatin pattern.

The human lymphocyte was observed to undergo the same cytologic changes as the rabbit lymphocyte, i.e., intranuclear vacuolization after 1000 r or less and death by delayed fixation after 2000 r or more. The recently developed slide chamber method[51] was used to study the rate of death of irradiated and nonirradiated human lymphocytes. According to this method, the in vitro longevity or the 10 per cent survival time of nonirradiated human lymphocytes was found to be, on the average, 9.2 days After irradiation with 1000 r, the lymphocytes from 19 individuals had a 10 per cent survival time of from 1.1 to 2.2 days with an average of 1.7 days.[52] Irradiation even with 5 r decreased appreciably the 10 per cent survival time. These experiments show that human lymphocytes are highly radiosensitive and that there is relatively slight variability in the sensitivity of the lymphocytes from different individuals

Tests were also made on the blood lymphocytes of 31 patients with chronic lymphocytic leukemia or lymphosarcoma with leukemia[54] The nonirradiated leukemic lymphocytes had an average 10 per cent survival time of 10 days The lymphocytes after irradiation with 1000 r had 10 per cent survival times which varied from 0 3 to 10 days It is particularly important to observe that 7 of the 31 leukemic patients had lymphocytes with 10 per cent survival times greater than 2 5 days In other words, about 20 per cent of the patients had blood lymphocytes that were resistant to x-rays The significance of the radioresistance of the lymphocytes in these patients is still under study

It was surprising to find that the leukemic lymphocytes of most of the patients had approximately the same radiosensitivity as normal lymphocytes Block[5] studied the effect of x rays in patients with leukemia or lymphoma by means of serial biopsies of the spleen He concludes, "There is no evidence to support the commonly held belief that malignant cells are more sensitive to x-irradiation than the corresponding nonmalignant cells" The in vitro and the in vivo findings are in accord

It is necessary to consider the radiosensitivity of lymphocytes of different species in order to know to what extent animal findings can be extrapolated to man The problem is complicated by the surprising finding that the 10 per cent survival time for the nonirradiated lymphocytes of the human is

9.2 days while that for the rat is only 2.1 days.[30] Irradiation with 10 r reduced the 10 per cent survival time both of the rat and the human lymphocytes. The lymphocytes of both species have approximately the same sensitivity to small doses of irradiation. However, the 10 per cent survival time of the human lymphocytes irradiated with 1000 r was 1.7 days, while that of the rat was only 0.1 days. The slower destructive action of irradiation on human cells is dependent on the difference in the longevity of the lymphocytes of the two species.

I have mentioned the theory that irradiation does not kill lymphocytes but accelerates the aging and death of the cells. I developed this concept as a result of studies by dark field microscopy on the death of irradiated and nonirradiated lymphocytes[47] and as a result of a comparison of the radiosensitivity of human and rodent lymphocytes.[53] In reviewing the literature for this paper, I find that in vivo studies have led other workers to express similar theories. Isaacs in 1932 stated that irradiation causes acceleration of maturation, senility, and death of blood cells.[29] And prior to that, in 1926 Nemenow[38] stated that in the ovary and other tissues the irradiated cell "both ages and perishes more rapidly, but it perishes exactly in the same way as it would have perished if it were to die, so to say, of a natural death." The theory does not answer the questions: what is the normal physiologic aging and death of the lymphocyte and how does irradiation accelerate these factors?

The finding that the lymphocyte has about the same sensitivity as the mitotic cell has led me to speculate that the lymphocyte and the mitotic cell have a similar metabolic process which is radiosensitive.[53] The radiosensitivity of cells in mitosis has been considered by many workers to be due to inhibition of the synthesis of desoxyribonucleic acid. The synthesis of DNA may be an important function of the lymphocyte, and interference with this function by x-rays may cause cell death.

IN VIVO STUDIES

Four different mechanisms have been proposed by various workers for the in vivo reactions of lymphocytes to x-rays. First, the irradiation may affect the lymphocytes by direct action on the cells. Second, the irradiation may inhibit the production of lymphocytes by acting on cells in mitosis. Third, the lymphocytes may be destroyed by indirect, humoral, or toxic effects induced by the irradiation. Finally, irradiation may be similar to other modes of stress which stimulate the adrenal glands to secrete cortical hormones deleterious to lymphocytes.

The indirect or humoral action of x-rays on lymphocytes has been suggested by clinical observations that irradiation of the spleen or of one group

of lymph nodes may be followed by lymphopenia and a decrease in size of other lymph nodes. The question of indirect effects has been critically reviewed by several investigators[10] who concluded that indirect effects have not been definitely proved and are probably not an important factor.

In this review I shall assume the point of view that the chief action of x-rays on lymphoid tissue in vivo is twofold: (1) a direct, destructive action on lymphocytes and (2) an inhibitory action on the production of these cells.

Under proper conditions four stages of reaction are observed in response to irradiation: (1) an initial, acute destruction, (2) an abortive recovery, (3) chronic lymphocytic depression, and (4) recovery.

INITIAL DESTRUCTIVE STAGE

The early effects of irradiation on lymphocytes were studied by Ross, Furth, and Bigelow[45] who collected the lymphatic fluid from the thoracic duct of rats before and after irradiation. Three to eight hours after irradiation of the whole animal with 750 r, the lymphcytes were found to have various denegerative changes including small vacuoles in the nucleus and cytoplasm, binucleated and multinucleated cells, cells with "lumped" chromatin, and finally dead cells with pyknotic and fragmented nuclei. By ten hours most of the injured cells and debris had disappeared, and the number of lymphocytes were reduced. It would seem that most of the lymphocytes died in vivo by the development of intranuclear vacuoles and pyknosis In addition, the binucleated and multinucleated cells and cells with lumped chromatin may be cells that were dying by delayed fixation. This and other studies indicate that irradiated lymphocytes undergo the same two types of degenerative changes in vivo as in vitro, i e , death by intranuclear vacuolization and death by delayed fixation.

Lymphoid Tissue. The first change in irradiated lymphoid tissue of animals is degeneration and death of lymphocytes as indicated by pyknosis and fragmentation of nuclei This change is seen after total body irradiation or after local irradiation of lymphoid tissue. Some of the degenerating *lymphocytes have intranuclear vacuoles*[1, 43, 61] *Irradiation of rabbits and* other animals with 25 r produces a perceptible effect on lymph nodes The destruction of lymphocytes with the formation of cellular debris is seen particularly in the secondary centers of lymphoid follicles of lymph nodes, in the cortex of the thymus, and in the Malpighian bodies of the spleen A lesser amount of necrotic debris is seen in the medullary cords of lymph nodes and in the medulla of the thymus The dead cells and the cellular debris are rapidly phagocytized by macrophages The debris-laden cells are seen almost as soon as degeneration occurs and persist for about 24 hours in the rabbit.

After total body irradiation of rabbits with 100 r, many lymph follicles persist more or less intact. After 600 r, the lymph follicles completely disappear. De Bruyn emphasizes the differences in the reactions following low dosages of 100 r or less as compared with high dosages of 600 and 800 r.[11] The destruction of lymph follicles may represent only an intensification of the process of lymphocytic degeneration, or perhaps it represents a new, qualitatively different effect produced by high doses of x-rays

In spite of the rapid death of the lymphocytes, the reticular cells in lymphoid tissue are resistant to large doses of x-rays,[41] even though the reticular cells are believed to be the precursors of lymphocytes. Similarly, some undifferentiated cells in the testis and ovary are more resistant to x-rays than the daughter cells The cells of the lymphocytic series are not unique in being exceptions to the law of Bergonié and Tribondeau that undifferentiated cells are radiosensitive

During the period of degenerative changes, mitoses are absent or greatly reduced in the lymphoid tissue.[27] In one study the number of mitotic figures decreased from 100 to 58 per unit volume of lymph node in one hour after irradiation of mice with 35 r[23]

In the spleen of patients, lymphocytes showed karyorrhexis and cessation of mitosis, 24 to 48 hours after irradiation[5] The cells undergoing karyorrhexis after a single depth dose of over 100 r were apparently fewer than in lower animals, possibly due to a slower rate of destruction of the human lymphocytes The reticular cells were resistant to the treatment. In lymphoid tissue the follicles were partly destroyed three to four weeks after irradiation of cancer of the cervix[47]

Autopsies of Japanese men and women who died from irradiation during the first 11 days after the atomic bomb attack showed a complete or almost complete absence of lymphocytes and of lymph follicles[31] The Malpighian bodies of the spleen were only vaguely outlined by whorled structures of collagenous and reticular tissue Phagocytosis of nuclear debris was seldom observed although erythrophagocytosis was present in the spleens The reticular cells were resistant to a dose of irradiation that was lethal to the individual Similar findings were obtained in a man who died 10 days after an accidental exposure to about 880 r x-rays[14] In general, the early changes observed in lymph nodes of human subjects after lethal total body irradiation were similar to those observed in irradiated animals

The quantitative reaction of lymphoid tissue to x-rays in vivo has been studied only by a few investigators Trowell counted the number of necrotic cells in lymph nodes of rats and obtained time-effect and dose-effect curves[59] The dose-effect curves were linear on probit paper when the dosages were 35 to 630 r Biagini obtained similar curves and in addition observed that a second x-ray treatment produced the same quantitative effect as the first[4]

Unfortunately quantitative data for other species are not available, although such curves are essential to extrapolate data to the human species

Vogel and Ballin determined the weight of the rat thymus 24 hours after irradiation with varying dosages.[62] After 400 r the decrease in weight was 59 per cent. After 30,000 r the disease was only 10 per cent. The larger dose produced less effect than the smaller. In addition, they observed that fewer pyknotic nuclei were found in the thymus 24 hours after irradiation with 20,000 r than after 100 r. A similar histologic finding was obtained by Trowell, Corp, and Lush for the thymus but not for lymph nodes.[60]

These interesting and paradoxical findings in vivo may be explained by the in vitro observation that higher doses of x-rays (2,000 r or more) produced death by delayed fixation[70] while lower doses such as 400 r produced death by intranuclear vacuolization and pyknosis[40] The decrease in the weight of the organ depends not on the death of the cells but on the removal or absorption of the dead cells by phagocytosis and autolysis. In the thymus, phagocytosis and removal of pyknotic nuclei are slower than in the spleen.[36] The fixed cells produced by the irradiation of the thymus with 20,000 r are probably less readily absorbed than the cells with pyknotic nuclei produced by 400 r.

Blood Lymphocytes. Associated with the rapid destruction of lymphocytes in the lymphoid tissue is a rapid decrease in the blood lymphocytes following radiation. In rabbits given total body irradiation, the lymphocyte count decreased rapidly and reached a minimum count in 24 to 48 hours (Fig 10-8).[46] In men who were accidentally exposed to total or almost total body irradiation, the lymphocyte count decreased more slowly, and it took one to five days for the count to reach a minimum[16] Accidental total body exposure of 64 individuals to 175 r reduced the lymphocyte count to 25 to 55 per cent of the control in three days.[10]

Lymphopenia also developed after therapy of patients for cancer with x-rays or radium[15, 25] The lymphopenia developed irrespective of which part of the body was irradiated Surface irradiation, however, with beta rays had no effect on the blood cells.[44] The important factors in the production of lymphopenia after therapy were the dose and the volume or weight of the part of the body irradiated Therefore some workers advocate the use of the integral dose which is defined as the dose multiplied by the estimated weight of irradiated tissues A dosage of 7 to 10 megagram roentgens was found to depress the lymphocyte count to 25 per cent of the original.[24]

Price and others analyzed mathematically the rate of fall of lymphocytes in irradiated humans and animals[42, 46] According to Goodfellow,[15] the rate of decrease of lymphocytes varied directly with the dosage of radium. However, treatment with 10,000 mgh or more produced a maximum 11 per cent daily decrease during the first 7 to 10 days of treatment.

Most workers report that 25 r are the minimum total body dose required to produce an appreciable decrease of lymphocytes in individual animals[21] In rabbits, for example, 25 r produced a decrease of about 25 per cent in the lymphocyte count in 24 hours. A statistically significant reduction of lymphocytes followed irradiation of groups of mice with 10 r according to Dougherty and White[14] and of rats with 5 r according to Ingram and Mason.[19]

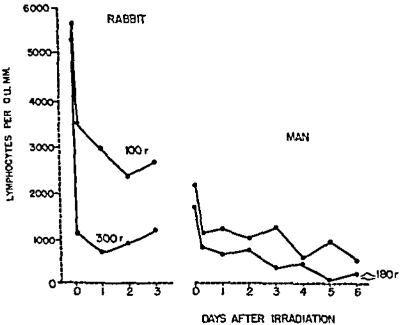


FIG 10-8 Early effects of total body irradiation on the number of lymphocytes in the blood (Data on rabbits from Sacher and Pearlman[16] and on man from Hemplemann, Lisco, and Hoffman [15])

In four patients treated with a total body exposure of 60 r, the lymphocyte count dropped in one day to 31 to 61 per cent of the original count.[10] One patient treated with 27 r and three normal individuals treated with 3 daily doses of 7 r did not develop any appreciable decrease in lymphocyte count A significant lymphopenia developed in patients who received total body irradiation of two daily doses of 20 r each.[32] The degree of lymphopenia after irradiation with varying dosages of x-rays was of the same order of magnitude in man, rat, and rabbit (Fig 10-9)

According to Cronkite's summary, an acute total body exposure of 25 r usually produces no definite lymphopenia in man, of 100 r results in a mild lymphopenia, and of over 100 r causes a lymphocyte count as low as 10 per cent of the normal in 24 hours.[9]

Degenerating and necrotic lymphocytes were seen in the blood smears of two men who received an acute body exposure of 290 and 880 r.[16] The dying and dead cells showed patchy condensation of chromatin, intranuclear vacuoles, and cytoplasmic basophilic granules and vacuoles. An occasional lymphocyte had a pyknotic nucleus. The degenerating cells continued to be present until the time of death, 24 and 10 days after irradiation In patients who received therapeutic irradiation, as many as 20 per cent

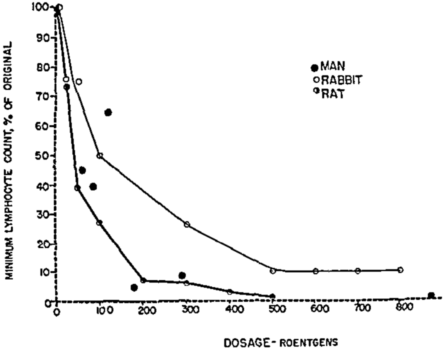


FIG 10 9 The relationship between dosage of total body x irradiation and minimal blood lymphocyte count in rat, rabbit, and man (Data from Sacher and Pearlman,[40] Hemplemann, Lisco, and Hoffman,[16] and Nickson.[40])

of the white blood cells were degenerating during the first 2 weeks after x-rays or radium therapy[34] Goodfellow[15] observed cells with bizarre-shaped nuclei and also rare cells with intranuclear vacuoles. He called these cells "monocytes," although he and others report great difficulty in classifying these abnormal cells The degenerating lymphocytes in blood smears are not specific for radiation but are also seen after administration of nitrogen mustard

In rats exposed to the sublethal dose of 400 r, pyknotic lymphocytes appeared in the blood and reached a peak of 7 per cent of the lymphocytes at three hours after irradiation and then declined rapidly.[50] In normal rats

about 0.2 per cent of the lymphocytes were found to be pyknotic in blood smears. It is of interest that the pyknotic cells were present only during the first few hours after irradiation in the rats but were found for many days in irradiated men.

Stage of Abortive Recovery

Blood Lymphocytes. Taylor, Witherbee, and Murphy[59] observed that irradiation of a monkey, a cat, and a pony caused a minimal lymphocyte

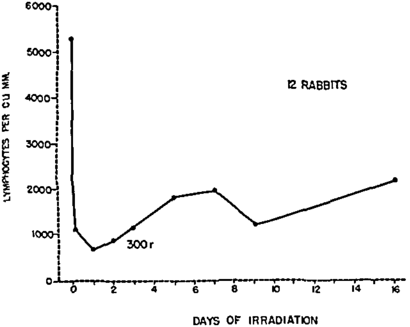


Fig. 10-10 Effects of total body irradiation with 300 r of x rays on the number of lymphocytes in the blood of rabbits (Data from Sacher and Pearlman[46])

count in 48 hours and then a primary rise in 3 to 9 days followed by a secondary fall from 5 to 12 days after the irradiation Jacobson and associates[22] observed a transient rise in the lymphocyte count following the initial lymphopenia in rabbits given a total body exposure of 200 r or more (Fig. 10-10) The rise occurred from 4 to 11 days after irradiation and was followed by a decline of the lymphocyte count in a few days even though the animal received no additional irradiation. The temporary rise in blood lymphocytes was called by Jacobson "abortive rise" or "recovery," and he has reviewed its occurrence in several species[21] Ross, Furth, and Bigelow observed an abortive rise in the number of lymphocytes in the thoracic

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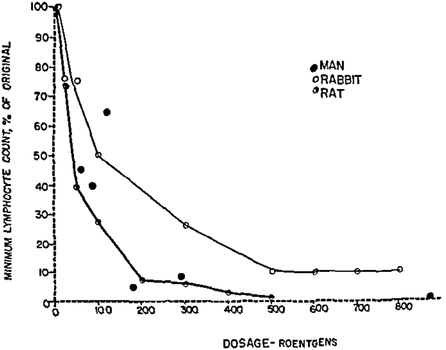


FIG 10-9 The relationship between dosage of total body x-irradiation and minimal blood lymphocyte count in rat, rabbit, and man (Data from Sacher and Pearlman,[46] Hemplemann, Lisco, and Hoffman,[18] and Nickson [49])

of the white blood cells were degenerating during the first 2 weeks after x-rays or radium therapy [34] Goodfellow[15] observed cells with bizarre-shaped nuclei and also rare cells with intranuclear vacuoles He called these cells "monocytes," although he and others report great difficulty in classifying these abnormal cells The degenerating lymphocytes in blood smears are not specific for radiation but are also seen after administration of nitrogen mustard

In rats exposed to the sublethal dose of 400 r, pyknotic lymphocytes appeared in the blood and reached a peak of 7 per cent of the lymphocytes at three hours after irradiation and then declined rapidly.[59] In normal rats

or clumped chromosomes. Henshaw[17] described multinucleated giant cells from 1 to 72 hours after irradiation of mice with 50 r. Abnormal cells or mitoses in lymphoid tissue of irradiated animals were also described by Murray[35] and Nettleship.[39] In irradiated tissue cultures of chick fibroblasts, Lasnitzki[28] observed two transient increases in the numbers of abnormal mitoses (Fig. 10-12). The maximum numbers occurred at 1 and 15 hours after irradiation with 100 r. In 18 hours the cultures showed normal mitotic activity and recovery.

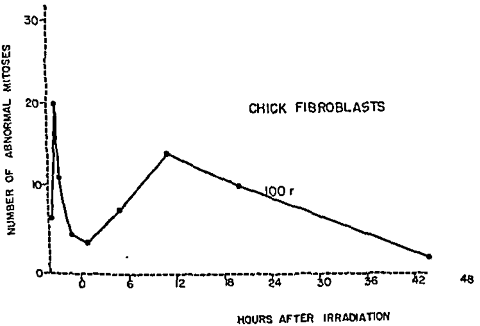


FIG 10 12 Effect of irradiation with 100 r of x rays on the number of abnormal mitoses in tissue cultures of chick fibroblasts (Data from Lasnitzki [28])

If abnormal mitoses are a criterion, irradiated human lymphoid tissues undergo a stage of abortive recovery In two individuals that died 10 and 21 days after accidental irradiation, the medium-sized lymphocytes in lymph nodes had many abnormal mitoses with chromosomal aberrations [18] Furthermore, many of the daughter lymphocytes were abnormal and many dividing cells died in mitosis. Many of the daughter lymphocytes had large hyperchromatic or multiple nuclei with the formation of some giant cells Many of the small lymphocytes had double nuclei or an unusually large amount of basophilic cytoplasm In Japanese men and women who died from 3 to 14 days following the atomic bomb attacks, the spleen and lymph nodes

duct of rats exposed to 750 r of total body irradiation.[45] The transient rise occurred from 5 to 7 hours after irradiation. In one patient with total body irradiation of 87.5 r in eight daily treatments, a suggestive abortive rise was observed from 17 to 30 days after therapy followed by a secondary decrease (Fig. 10-11).[40]

Lymphoid Tissue. In lymphoid tissue abortive recovery is characterized by a transient increase in mitoses following the initial inhibition. Barrow and Tullis[1] described a temporary return of mitotic activity in the white

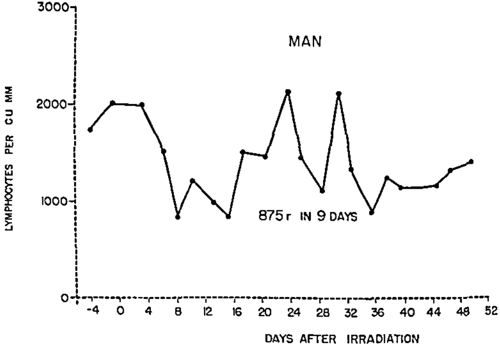


FIG. 10-11 Effects of total body irradiation with a total of 87.5 r in 9 days on the blood lymphocyte count in one man (Data from Nickson [40])

pulp of the spleen 10 to 36 hours after irradiation of mice with 1000 r A similar wave of regeneration has been described in myeloid tissue by Bloom.[6] Actual counts of mitotic figures were done by Knowlton and Hempelmann who observed an abortive rise in the number of mitoses in the adrenal gland and jejunal mucosa but not of the lymphoid tissue, possibly due to insufficiently close time intervals[23] In spite of the difficulties of estimating the number of mitoses and in spite of the resulting paucity of reports, it seems that lymphoid tissue undergoes abortive regeneration

In the spleens showing abortive regeneration, Barrow and Tullis observed many abnormal mitosis, such as tripolar and multipolar figures, and lagging

atypical lymphocyte, similar to that seen in infectious mononucleosis, was observed in survivors of the atomic bomb attacks.[41]

STAGE OF RECOVERY

Lymphoid Tissue. The fourth stage in the radiation reaction is the stage of recovery. This stage is associated with a gradual increasing number of mitoses in reticular cells and in medium sized lymphocytes[11] The mitotic cells were observed not only in the germinal centers of persisting follicles but also diffusely throughout the node. The lymph node appeared histologically normal five days after irradiation of rabbits with 100 r.

The recovery was slower in lymph nodes of rabbits exposed with 600 or 800 r. In these nodes the lymph follicles had been largely destroyed. Early diffuse regeneration was first observed 5 days after irradiation with gradual repopulation of lymphocytes by mitoses of medium-sized lymphocytes Nodular regeneration started 21 days after irradiation with production of small, "bare" germinal centers consisting of medium-sized lymphocytes, some in mitosis. The lymph node was reconstituted in 59 days in rabbits exposed to 600 r. This may be contrasted to the rapid regeneration in 5 days of lymph nodes of rabbits exposed to 100 r

Abdominal irradiation with 400 r in rabbits resulted in lymphoid hyperplasia in the omentum with the production of many new lymph follicles[33] Overcompensation of mitotic activity was observed in the lymphoid tissues of mice[21] and of chick embryos.[61] In spite of its radiosensitivity, lymphoid tissue recovers rapidly after moderate doses of x-rays and shows a tendency to hyperplasia and overcompensation of mitotic activity

In Japanese individuals dying three or more weeks after the atomic bomb attacks, the spleen had small lymphocytes and occasional mitotic figures in syncytial, spindle-shaped reticulum cells near the central arterioles[31] Early follicles developed with a peripheral "germinal center" similar to the "hematopoietic peri-follicular envelop' seen in the spleen of rats by Krumbhaar[26] In lymph nodes, formation of germinal centers was observed in only one patient after the sixth week In seven patients irradiated for cancer of the cervix, the lymph nodes showed a reduced number of follicles three to six months after irradiation[67] The findings indicate that regeneration of follicles is slow after heavy irradiation

Blood Lymphocytes. Recovery of the number of lymphocytes in the lymph of the thoracic duct started two weeks after irradiation of rats with 750 r[45] During the regenerating phase as many as 70 per cent of the white cells were large lymphocytes as compared to 2 to 5 per cent in non-irradiated animals Many of the large lymphocytes were abnormal and had lobed, eccentric, or multilobed nuclei The number and the morphology of

showed the proliferation of atypical cells.[31] These were variable in appearance and resembled reticulum cells, lymphoblasts, "splenic tumor cells" of Rich, Lewis, and Wintrobe, and some even resembled Reed-Sternberg cells of Hodgkin's disease. The atypical cells gradually became less common in spleens of patients dying six weeks after irradiation.

Abortive recovery occurs in many types of cells. The best description and explanation are given by Puck and Marcus.[43] They found that an isolated HeLa cell irradiated in vitro with 600 r usually underwent four or five divisions with the production of giant and other atypical cells which could not divide. Presumably a similar process occurs in irradiated lymphoid tissue and produces the stage of abortive recovery. It is not known whether the abnormal mitoses and atypical cells in irradiated lymphoid tissue have any relation to the development of postirradiation leukemia.

Chronic Morphologic Changes in Lymphocytes

Various types of abnormal lymphocytes may appear in the blood. These morphologically altered lymphocytes differed from the degenerating cells which develop during the acute destructive stage although the differentiation is not always clear-cut. Dickie and Hempelmann observed an increase in the number of refractile neutral red granules in supravitally stained lymphocytes of individuals who received 1.5 r per month.[12] Watts and Mathieson, however, were not able to find an increase of lymphocytes with refractile cytoplasmic granules in the blood of persons treated with radioactive iodine.[65]

Ingram and colleagues[18] found in blood smears of cyclotron workers a few lymphocytes with bilobed and double nuclei. The workers had received exposures below the tolerance dose. Differential counts showed that the workers had 7.5 binucleated lymphocytes per 50,000 white blood cells as compared to 1.5 cells per 50,000 in control individuals. These cells were considered to be the result of abnormal mitoses. In persons exposed to 0.2 r per week, the number of binucleated lymphocytes were increased to 5.9 per 50,000 white blood cells as compared to 1.4 per 50,000 in control individuals.[13] These lymphocytes were also observed in mice exposed to only 2 r. Lower doses were not tested. The incidence of these cells did not depend on the dosage of irradiation between 2 to 75 r. The binucleated lymphocytes are the most sensitive indicator of radiation of lymphoid tissue, but the cells are not specific for irradiation. Various other types of abnormal cells have been described in irradiated men and animals: lymphocytes with clover-leaf-shaped nuclei,[2] lymphoblasts, cells difficult to differentiate from monocytes,[15] and even isolated and clumped reticular cells.[29] An unusually

X-rays, however, had a relatively slow destructive action on the human lymphocytes. The in vitro and the in vivo findings are in accord

SUMMARY AND CONCLUSIONS

Moderate doses of irradiation accelerate the aging of normal lymphocytes with death by intranuclear vacuolization and pyknosis Large doses of irradiation result in death by delayed fixation. These two degenerative changes occur both in vitro and in vivo in man and in lower animals Irradiation also causes early inhibition of mitoses. These radiation effects result in the initial acute destructive stage In the lymphoid tissue this stage is characterized by (1) rapid destruction of lymphocytes, (2) phagocytosis of the necrotic debris, and (3) no perceptible reaction of the reticular cell to the irradiation. In the blood there is a slight or a severe lymphopenia and the temporary appearance of degenerating and dead lymphocytes

Another effect of irradiation is a transient increase in abnormal mitoses which results in the stage of abortive recovery This stage is characterized by a temporary alleviation of the lymphopenia, by a transient increase of normal and abnormal mitoses, and by the appearance of abnormal cells in lymphoid tissue. The stage of chronic lymphocytic depression is the result of a low level of production of lymphocytes but no increased destruction Finally, the recovery stage is associated with more or less normal lymphopoiesis, although there is probably some persistent abnormal production giving rise to a small number of abnormal lymphocytes

I have reported in this chapter that the in vitro and in vivo findings are in accord on the following points (1) Irradiation causes death of lymphocytes by intranuclear vacuolization after low dosages and by delayed fixation after high dosages (2) The lymphocytes of man and lower animals have approximately the same radiosensitivity (3) Human lymphocytes react more slowly to x-rays than do rat lymphocytes (4) In general, human leukemic and normal lymphocytes have the same degree of radiosensitivity (5) The irradiated cell undergoes abnormal mitoses with the production of atypical cells.

An enormous amount of work has been done in the field of the biologic effects of irradiation. The subject is however, still in an active state of growth, but there are still many obvious gaps in our knowledge. This study has led me to the belief that there is a need for additional quantitative data both in vivo and in vitro When such data are available, they will provide a means for a better understanding of the fundamental reactions of the lymphocyte to x-rays and for a better understanding of the lymphocyte itself.

the lymphocytes returned to normal in about 30 days after irradiation.

After a single total body exposure of rabbits, the lymphocyte count returned to normal in 2 days after 25 r and in 50 days or more after 400 r of total body irradiation.[21] In contrast it took only 9 days for the granulocyte count to return to normal after 500 r. The lymphocyte is the first cell to *degenerate after total body irradiation and the last to return to normal.* The animals had no compensatory absolute lymphocytosis after acute total body irradiation.[7]

Rabbits irradiated with 400 r show recovery of the histologic structure of lymphoid tissue in 5 days and the recovery of the number of blood lymphocytes in about 50 days. Recovery is not as rapid in blood lymphocytes as in lymphoid tissue.[8]

The slowness of the return of the normal lymphocyte count is especially prominent in man. After total body irradiation with only 60 r, the lymphocyte count did not return to normal even after 5 months in two of four individuals.[40] Two individuals who were accidentally irradiated continued to have lymphopenia 1 to 2 years after the accident.[16] Two years after a high dosage of total body exposure from the Hiroshima atom bomb, survivors had a slightly lower lymphocyte count than a control population.[56] After therapeutic irradiation with x-rays or radium, the recovery period was 1 to 19 weeks with an average of 5 weeks in one study[25] and at least from 4 to 6 weeks in another.[17]

Species Effect

According to Jacobson and associates,[22] man shows the same degree of radiosensitivity of lymphocytes as the guinea pig and the dog. The qualitative changes in the blood are the same in man and lower animals.[9] The chief difference in the hematologic response of man and animals is in temporal relations.[37] After total body irradiation with about 400 r, the lymphopenia developed within 2 days in the rabbit and within 5 days in man. The abortive rise of blood lymphocytes was observed in from 4 to 11 days in the rabbit and at from 17 to 30 days in one man. Lymphoid tissue showed abortive recovery in 10 to 36 hours after 1000 r in mice and probably in 3 days to several weeks in man after lethal irradiation. Recovery of the normal lymphocyte count occurred in about 2 months in rabbits after a large dose of x-rays, but it took 2 years or more for a man to recover. An irradiated man takes a longer time to reach minimal leukocytic levels and to recover normal levels than irradiated laboratory animals.

According to the in vitro findings that I have described, the lymphocytes of men and lower animals have approximately similar radiosensitivities

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CHAPTER 11

NONLEUKEMIC LYMPHOCYTOSIS: *Diseases and Mechanisms*

JOHN B. MIALE

Qualitative and quantitative changes in the lymphocytes of the peripheral blood are important in recognizing diseases accompanied by specific or non-specific lymphocytic reactions.

DEFINITION AND SIGNIFICANCE OF LYMPHOCYTOSIS

Regardless of the nature and etiology of the disorder, it is important first to define the term "lymphocytosis" Lymphocytosis, as are other leukocytoses, is classified as either *relative, absolute*, or a combination of the two. *Relative lymphocytosis* is a relative increase in the percentile proportion of lymphocytes to other leukocytes in the peripheral blood as determined by the differential count *Absolute lymphocytosis*, on the other hand, is defined as an increase in the absolute number of circulating lymphocytes and is determined by multiplying the percentage of lymphocytes from the differential count by the leukocyte count per cubic millimeter of blood Since relative percentile counts express only the ratio between the various leukocytes in peripheral blood, it is obvious that relative lymphocytosis may or may not have any significance Thus, when relative lymphocytosis is due to a decrease in other cells, particularly the neutrophils, the important change is the neutropenia rather than the apparent increase in the lymphocytes On the other hand, when a relative lymphocytosis is also accompanied by a higher than normal leukocyte count, the relative lymphocytosis is an expression of the increased number of circulating lymphocytes and becomes significant Unless, then, the total leukocyte count is known, it is difficult to attach any importance to relative values alone

The significance of absolute values can only be interpreted in the light of *established normals* The number of circulating lymphocytes per cubic millimeter of blood varies at different ages and thus different normal values can be established for various age groups (Fig 11-1). In accordance with these normal values, absolute lymphocytosis occurs when there are more than 1000 lymphocytes per cubic millimeter of blood in adults, 7000 per cubic millimeter in children, or 9000 per cubic millimeter in infants and young children.

CONDITIONS ACCOMPANIED BY LYMPHOCYTOSIS

The conditions which are accompanied by (nonleukemic) lymphocytosis can be classified in two general groups (Table 11-1) In Group I are diseases in which lymphocytosis is either so characteristic in type or so constant in occurrence as to have diagnostic importance. In Group II are diseases that may, at some stage, show lymphocytosis, but this is transient, inconstant, and by itself not particularly diagnostic

The lymphocytic responses which accompany the conditions listed in Group II are seldom striking in degree. If one follows carefully the peripheral blood picture in hospital patients, it is not uncommon to encounter transient lymphocytosis in a great variety of diseases. It was felt at one time that the lymphocytosis seen during the recovery phase of many infectious diseases is of prognostic significance Although much was made of this in the older literature, it is my feeling that one can no longer attach much significance to this change. This is not to negate the older findings. Rather, the course of infectious diseases has been markedly altered in the last 20 years by the use of antibiotics, with altered responses and accelerated recoveries, so that one depends on more reliable criteria to determine prognosis and the course of a disease

Turning then to Group I, it is apparent that these diseases have either a proved or almost certain viral etiology It seems to me that there is some merit in further dividing this first group into two subgroups Subgroup I-A is headed by infectious mononucleosis and is composed of viral diseases in which the peripheral blood smear shows "atypical lymphocytes" We have called these atypical lymphocytes "virocytes" in accordance with the suggestion made by Litwins and Lebowitz [12] Subgroup I-B is characterized by the appearance in the peripheral blood of a larger than normal number of lymphocytes which generally are normal in appearance, usually of the small type, and which do not have the morphologic features of virocytes

I should like first to dispose of the second group by saying that lymphocytosis is a common and reliable feature of the peripheral blood in these diseases Although it does not aid in distinguishing between diseases by

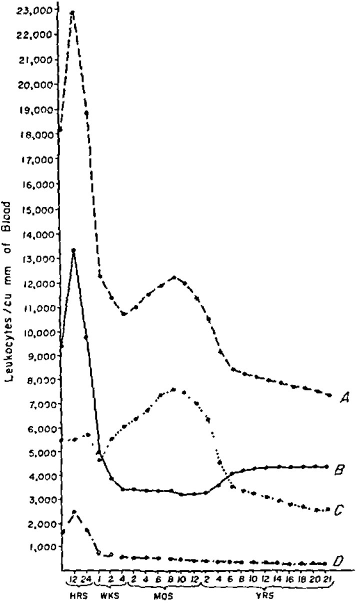


FIG. 11.1 Average normal leukocyte count and absolute numbers of circulating leukocytes at various ages. (*A*) leukocyte count, (*B*) circulating neutrophils, (*C*) circulating lymphocytes, (*D*) circulating monocytes. (From Miale, J. B.: *Laboratory Medicine—Hematology*. St. Louis, C. V. Mosby Company, 1958.)

Table 11-1. Conditions Accompanied by Lymphocytosis (Pathologic, Nonleukemic)

I. Constant or diagnostic
- A. Lymphocytes of the "virocyte" type
  1. Infectious mononucleosis
  2. Acute viral hepatitis
  3. Viral pneumonitis
  4. Herpes zoster
  5. Herpes simplex
  6. Roseola infantum
  7. Upper respiratory infections (viral)
- B. Lymphocytosis, normal morphology
  1. Infectious lymphocytosis
  2. Mumps
  3. Chicken pox
  4. German measles
  5. Pertussis

II. Transient or inconstant
- A. Congenital syphilis
- B. Secondary stage of syphilis
- C. Scarlet fever
- D. Brucellosis
- E. Typhoid fever
- F. Hyperthyroidism
- G. Myasthenia gravis
- H. Hypopituitarism
- I. Chronic tuberculosis
- J. Chronic exposure to ionizing radiation
- K. Others

itself, it is often helpful in corroborating the clinical diagnosis. At times the lymphocytosis is present in the prodromal stage before a clinical diagnosis is possible

This then leaves Subgroup I-A. If a grouping of this sort is justified, it raises some intriguing points. The common denominator of this group is the presence in the peripheral blood of mononuclear cells which are almost certainly of lymphocytic origin These cells do not fit the usual descriptions of either normal or of leukemic lymphocytes For want of a better term, they have often been referred to as "atypical lymphocytes" Attention was drawn to these cells as soon as hematologists began examining the peripheral blood For example, Turk in 1907[18] recorded a case in which the peripheral blood contained atypical cells thought to be blasts of acute leukemia but remarkable in that the patient recovered. It has been suggested that this is one of the earliest reported cases of what we now call infectious mononucleosis, or at least one of the earliest cases in which the peripheral blood was studied Turk's description of an atypical cell having cytoplasmic features of a plasma cell and a somewhat immature nucleus

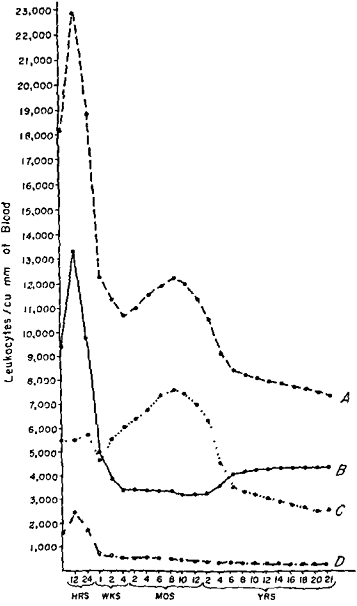


FIG 11 1 Average normal leukocyte count and absolute numbers of circulating leukocytes at various ages (*A*) leukocyte count, (*B*) circulating neutrophils, (*C*) circulating lymphocytes, (*D*) circulating monocytes (From Miale, J B *Laboratory Medicine—Hematology* St Louis C V Mosby Company 1958)

blood are so characteristic that a diagnosis of infectious mononucleosis can be made from the inspection of the peripheral blood smear alone. This statement is true only in a very limited sense

In the first place, cells which individually have the features of those seen in infectious mononucleosis are found in the peripheral blood in a variety

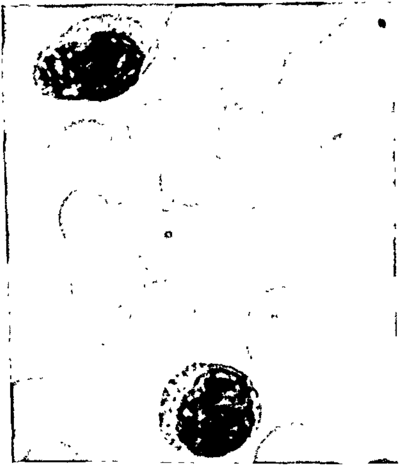


FIG 11-3 Infectious mononucleosis, peripheral blood Wright's stain (× 1700)

of conditions (Table 11-1, Subgroup I-A) and observation which has been recorded by a number of writers[2, 17, 16] and even by McKinlay and Downey.[13] Figures 11-2 and 11-3 show cells in smears of two proved cases of infectious mononucleosis The cell shown in Figure 11-4 is from the peripheral blood in a case of primary viral pneumonitis The presence in the peripheral

is not unlike that of cells which I shall illustrate later. To this day there is no general agreement as to what a Turk cell is,[15] and, since Turk's own description was hardly definitive enough to justify the eponym, we must blame his successors for establishing this term firmly in the literature and in laboratory slang



Fig 11-2 Infectious mononucleosis, peripheral blood Wright's stain (× 1700)

Infectious Mononucleosis

The paper of Evans and Sprunt[7] established the term "infectious mononucleosis" for the acute illness characterized by abnormal lymphocytes in the peripheral blood Three years later Downey and McKinlay[5] provided the classic description of atypical lymphocytes of infectious mononucleosis, classified these cells into three types, and thus became responsible for the concept that the atypical lymphocyte of infectious mononucleosis is so characteristic as to justify the term "infectious mononucleosis cell" It was only one small step from this to the concept that these cells in the peripheral

makes the peripheral blood smear in infectious mononucleosis characteristic if not diagnostic. In diseases other than infectious mononucleosis, virocytes seldom make up more than 15 per cent of the leukocytes. It is also true that the virocytes seen in conditions other than infectious mononucleosis usually have the appearance of the cells described by Downey as Type 3.

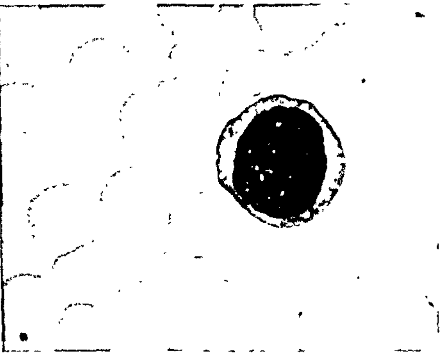


FIG 11 5 Herpes zoster, peripheral blood Wright's stain (× 1700)

However, the classification as to the type of virocyte in infectious mononucleosis has little significance since a rather rapid transition can be observed from one type to another if serial peripheral blood smears are examined. On morphologic grounds alone, then, the atypical lymphocytes seen in the peripheral blood in the conditions listed in Subgroup I-A are very similar and for this reason it seems justified to call them "virocytes" and to abandon the use of the term "infectious mononucleosis cell"

It might be said that since the diagnosis of infectious mononucleosis can be substantiated by serologic studies,[3] these morphologic observations are not particularly disturbing This is only partially true. In the first place the presence of virocytes can often be of great assistance in suspecting the diagnosis in diseases such as infectious hepatitis or viral pneumonitis when the

blood of similar cells has been recorded by others[16, 12] Figure 11-5 illustrates an atypical lymphocyte in the peripheral blood smear of a patient with herpes zoster.[12, 16] Figures 11-6 and 11-7 illustrate the atypical lymphocytes in the peripheral blood smear of patients with viral hepatitis. Litwins and Lebowitz[12] record the presence of similar cells in 53 per cent of a series of



FIG. 11 4 Primary viral pneumonitis peripheral blood Wright's stain (× 1700)

55 patients with infectious hepatitis which they studied, and similar reports have been made by others[1, 9, 10 12 14, 21]

Although it would have been possible to select cells not as alike as the ones illustrated, it must be admitted that, if the captions were intentionally omitted, it would be difficult to pick out which of these cases is infectious mononucleosis and which is not It is true that in general infectious mononucleosis exhibits a greater percentage of virocytes in the peripheral blood smear than do the other conditions listed This is one of the features that

in infectious mononucleosis. In many of these cases, the cells were also studied by phase microscopy and were found to have the same features as those of cells in proved cases of infectious mononucleosis.[14] However, serial serologic investigations revealed that only 4 of the 58 cases had antibodies which were not absorbed by Forssman antigen and were absorbed by beef



FIG. 11 7 Viral hepatitis, peripheral blood Wright's stain (× 1700)

erythrocytes Titres (after absorption) in these four cases were high and definitely diagnostic (1:112, 1:448, 1 448, 1:896) In the other 54 cases there was little if any elevation of nonspecific heterophil antibody and no increase in heterophil antibody of the infectious mononucleosis type. We satisfied ourselves that these results were not due to technical difficulties There is no doubt in my mind that this represents a disease similar to, but not the same as, infectious mononucleosis. It is not likely, nor is it within the experience of others, that there should be an epidemic of serologically negative cases of infectious mononucleosis

clinical findings are doubtful. In the second place the diagnosis of infectious mononucleosis in my opinion must not be made unless three criteria are fulfilled: (1) a compatible clinical picture, (2) a compatible peripheral blood smear, and (3) typical serologic results showing the presence of antibody not absorbed by Forssman antigen but absorbed by beef erythrocytes

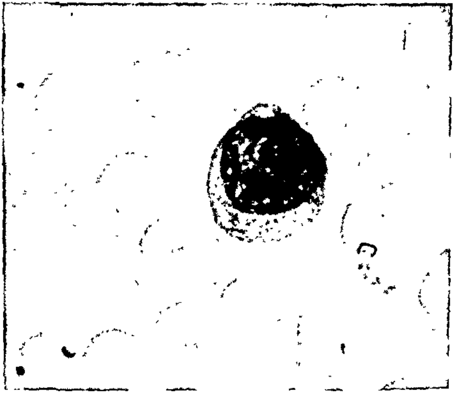


FIG 11-6 Viral hepatitis, peripheral blood Wright's stain (× 1700)

However, it is common experience that sometimes cases are encountered satisfying the first two criteria but not the third Many have recorded this,[4,6,8,20] blaming either the specificity of the test or the system of interpretation or technical factors Some have attempted to separate such an entity by the term "pseudomononucleosis"[19]

I have commented previously[14] on a group of 58 cases studied during the winter of 1956 in Miami During a period of three months, we followed carefully cases of an apparently epidemic disease which clinically had the features of infectious mononucleosis Serial studies on the peripheral blood showed numerous atypical lymphocytes, seldom below 50 per cent of the leukocyte count, cells which could be classified as one or the other of Downey's three types and which we thought morphogically typical of the cells seen

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MECHANISMS OF LYMPHOCYTOSIS

The following discussion of the mechanism of lymphocytosis is necessarily brief because Chapters 1 and 5 deal directly or indirectly with mechanisms Much that has been written on this subject is conjecture and does not bear repeating One of the fundamental laws of hematology is that the number of cells occurring at any given time in the peripheral blood are determined by the net effect of three processes (1) the number of cells produced in the responsible hemopoietic tissue, (2) the rate at which these cells are released into the blood stream, and (3) their life span. If we attempt to apply critically these criteria to an elucidation of the mechanism by which lymphocytosis is produced, we soon find that we are floundering in a sea of ignorance or controversy.

For example, there can be little argument with the concept that lymphocytes are produced exclusively in lymphoid tissues. In addition to lymph nodes, lymphoid tissue is widely scattered throughout the body in organs such as the spleen, bone marrow, and gastrointestinal tract, and also in a diffuse fashion in many other locations. Certainly one route by which lymphocytes enter the blood stream is by way of the thoracic duct, but it is easier to imagine lymphocytes from the gastrointestinal tract finding their way into the thoracic duct and the venous blood than to picture the migration of lymphcocytes through a series of lymph nodes into the collecting lymphatic vessels and then into the blood stream It is probable that lymphocytes also enter capillaries directly without being transported by the thoracic duct, but I know of no data to indicate whether or not this is true and, if so, what the relative proportion is of lymphocytes entering the blood stream by various routes We obviously do not know the rate at which these cells are released from the lymphopoietic tissues. At one time it was thought that a count of the number of lymphocytes per cubic millimeter of lymph in the thoracic duct gave a measure of the total number of lymphocytes entering the blood stream and that from this estimates of their life span could be made More recent work indicates that one needs to revise radically old concepts about the life span of lymphocytes, their biologic behavior, and their fate, also the manner by which they enter and leave the blood stream, where they go, and how long they last at each location. At this time we must be satisfied to ask questions for which we do not know the answers

2. In general, leukemic lymphocytes that are morphologically similar to cells in some stage of normal lymphocytogenesis are more likely to be widely and diffusely disseminated with the clinical, hematologic, and pathologic features of leukemia than are tumors whose cells have abnormal nuclear or cytoplasmic deviations. The latter are more likely to be cases of lymphosarcoma.[3]

Exceptions: In numerous instances tumors with nuclear and cytoplasmic abnormalities not found in normal tissues may have the manifestations of a diffuse disease indicative of leukemia. Similarly, it is not always possible to detect morphologic abnormalities in cells of tumors with restricted growth.

It should be noted that the nuclear and cytoplasmic characteristics of the cells are studied to best advantage in smear or imprint preparations[6, 11] while the general architectural and topographic features of nodal structure are better demonstrated in sections Study of a lymph node is not complete unless both smear (or imprint) and sectioned material have been examined.

3 In general, cases with relatively mature cells in the blood and tissues are more likely to be chronic, while immature cells usually predominate in the more acute forms.

A parallel situation occurs in all tumors The procedure of "grading" tumors depends on determining the proportion of the more or less mature (differentiated) and immature (undifferentiated) cell types

Exceptions The degree of malignancy as indicated by the clinical course is not always that expected from study of the cells. Cases with relatively mature cells may progress rapidly or have acute exacerbations; cases with immature cells (lymphoblasts) may have courses longer than anticipated.

4 Tumors with a follicular histologic pattern (follicular lymphoblastoma, giant follicle lymphoblastoma) are likely to have a prolonged course. If the presence of a follicular pattern may be taken as an indication of maturation, this again is in conformity with the general statement that maturity is usually accompanied by a longer course

Exceptions The subsequent occurrence of obvious clinical malignancy, which usually occurs only after a long interval, is sometimes rapid in its development

5 Tumors composed of cells larger than a lymphocyte and with clearer nuclei and cell bodies are often referred to as reticulum cell tumors without further study of cell details. Size and cell clarity alone are not enough for the identification of reticulum cells (histiocytes) The sarcomatous tumors that have been described under this name comprise a heterogeneous group[3]

CHAPTER 12

LYMPHOCYTES OF THE LYMPHOCYTIC LEUKEMIAS

MAURICE N. RICHTER

This chapter is limited to certain aspects of the cells and lesions found in leukemia and related states, especially the relations of cell types, tissue changes, and clinical course.

Neoplastic conditions involving lymphocytes occur in forms which at one extreme are localized to certain regions and at the other extreme are widely disseminated, involving many, or seemingly all, lymphatic tissues The presence of intermediate and transition forms raises questions of probable relationships and reasons for the differences in distribution

CELL TYPES

Similarly, the cell types of tumors may be morphologically typical of some stage in the normal development of the lymphocyte or may deviate therefrom by having nuclear or cytoplasmic abnormalities not found in normal lymphopoiesis. *Although tumors arising from so-called reticulum cells or* histiocytes are not lymphatic tumors, they must be discussed because of the possible relationships of their cells of origin

Certain general statements, to which there are numerous exceptions, may be made

1 In general, tumors that apparently arise in individual lymph nodes or node groups tend to spread by metastatic deposits of more or less focal (localized, nodular) character, often in regional areas at first, in much the same way that metastases from other types of tumor occur

Exceptions In some cases the rapid dissemination of cells by the blood stream obscures the sequence of events, leads to diffuse involvement, and raises the question of possible multiplicity of foci of origin

greatest importance that when a practitioner sends an excised lymph node to the pathologist for diagnosis, he should at the same time send all the clinical information available. Too often the specimen is sent in without a word of history. This may be taken as subtle flattery for the omniscient pathologist, but it is actually an expression of culpable carelessness, and is grossly unfair to the patient who has entrusted himself to the practitioner."[1]

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with cells that may be large or small, mononuclear or multinuclear, and that may or may not be associated with the lesions of leukemia or Hodgkin's disease. In relation to the present discussion, it is probable that some of the tumors with cells of uniform size only slightly larger than lymphocytes may be composed of lymphoblasts[4, 12] or of primitive lymphoid cells (reticular lymphoblasts).

6 In general, the kind of cell and type of manifestation (focal or diffuse) tend to remain the same throughout the course of the disease.

Exceptions The exceptions are numerous[2] but are of limited kinds. Changes from a localized to a generalized form of disease or from a chronic to an acute type are not fundamental changes in kind, but in manifestation

Changes in cell type, for example from reticulum cell to lymphocyte or vice versa, are theoretically possible but are certainly exceptional The possibility of the coexistence of two neoplasms (e g., reticulum cell sarcoma and lymphocytic leukemia[9]) makes the interpretation of such cases on the basis of "change of cell type" difficult. On the other hand, changes within the concept of "Hodgkin's disease" (e g., change from Hodgkin's paragranuloma to granuloma to sarcoma) are not changes of kind, inasmuch as involvement of the same cell or origin (reticulum cell, histiocyte) is the basic lesion in all

LEUKEMIA IN MICE

A study of leukemia in mice provides information on a few points relative to this discussion

1 The blood picture and distribution of lesions of mouse leukemia transmitted by cell implants are not necessarily the same as in the original donor mouse,[10] indicating that these features do not indicate differences in kind

2 Changes in the distribution of lesions in transplanted leukemia may be affected by experimental procedures,[8] indicating that different manifestations of the disease are dependent in part on the relationship obtaining between the transplanted cells and the host

3. Changes in acuity of the disease during a series of transmissions may be preceded by changes in cell morphology[7]

SUMMARY

These observations indicate that study of the peripheral blood or of an excised lymph node does not alone enable one to determine the distribution of lesions or the rapidity of their course The importance of supplementary information is clearly expressed by Boyd. "It is therefore of the

Of historic interest is the experimental transmission of the canine venereal tumor from a naturally infected dog to other dogs, which was accomplished in 1876 by a Russian veterinarian, Novinsky This feat prompted at least one of Novinsky's countrymen to consider him "the forefather of experimental oncology." An important milestone in research on lymphocytic malignancies was passed in 1908 when Ellermann and Bang experimentally transmitted fowl leukosis from affected to normal chickens, although the disease had been described as a natural occurrence in birds as early as 1868.

Our purpose is to point out certain interesting features of this group of diseases in birds, swine, horses, rodents, monkeys, and cattle and to make more detailed comparisons between the dog and cat. (The disease of mice is discussed in Chapter 12.)

LYMPHOCYTIC MALIGNANCIES IN SPECIES

Birds

The term "fowl leukosis" is used to encompass the erythroid, myeloid, and lymphoid malignancies of the domestic fowl. The erythroid and myeloid forms are most readily induced experimentally in susceptible birds and have been the subject of rather extensive laboratory study. The lymphoid form of the disease is widespread in nature to the extent that it constitutes an important economic problem. However, it is more difficult to induce in the laboratory. Limitations of time preclude further consideration of this avian disease, although it presents many interesting similarities as well as differences in comparison with neoplastic lymphocytic disease in mammals[7, 11]

Pigs

Although some of the earliest reports on leukemia were devoted to swine, the disease does not appear to be common, at least to judge from the literature Swine are susceptible, however, and some well-documented cases are to be found[1a, 14, 18, 29]

Horses

Malignant lymphoma occurs in this species,[23] but its frequency is unknown Decreased interest in these animals over the past three decades has resulted in few studies of such a rare disease. It is possible that the present growing interest in horses and ponies may make studies in the equine species possible again

CHAPTER 13

COMPARATIVE PATHOLOGY OF LYMPHOCYTIC MALIGNANCIES

T. C. JONES and JERI A. BERG

Malignant neoplastic disease of lymphoid cells apparently occurs in all species in which these cells are recognized These species include all vertebrates, among which both mammals and birds are known to be susceptible. Although a great deal of information has been accumulated relative to lymphocytic malignancies in various species, no sustained effort has yet been made toward the thorough investigation of the disease in any species with the exception of chickens, mice, and men Still to be acquired is the most important data relative to this group of neoplastic diseases in lower animals Not yet are the data adequate for use toward understanding and controlling the human disease Intensive research in several species is greatly needed on this problem because the similarities in different animals point toward common or related etiologic factors, in spite of the complex and confusing nomenclature in present use which tends to obscure this important possibility

Present concepts of this disease complex appear to have started to develop from the work of Virchow, who in 1845 grouped human cases of malignant disease involving blood cells and introduced the term "leukamie" Similar disease was soon recognized in animals by Leisering[10] who described a case in a horse in 1858 and another in a pig in 1865 Bollinger reported cases in pigs and a dog in 1871, and Siedamgrotzky described cases in dogs and cats in that same year Eberth is credited with reporting the first case in a mouse in 1874, Jacob recorded the first case in an elephant in 1908 At the present time malignant lymphocytic disease has been recognized in all domestic and many wild species of birds and mammals The disease is not known in species below the vertebrates

It is of interest that Dobberman and Seifried[10] recorded the condemnation for leukemia of from 0.15 to 0.4 per cent of cattle slaughtered in Germany during the years 1927 to 1936. These authors also reported 100 per cent increase in the disease in the course of those years. Junack[10] in 1932 expressed an opinion that malignant lymphoma in cattle had doubled in frequency during the preceding 25 years. Lockau[10] in 1933 reported finding the disease in 0.6 per cent of 24,000 cattle slaughtered in Berlin. Schottler and Schottler[10] found the disease to affect 10 per cent of the animals in some herds in East Prussia.



FIG 13-1 Canine venereal tumor Large friable tumor mass on the penis of a dog (AFIP 732552)

Dogs

In the dog several disease entities must be considered by the pathologist in reaching a definitive diagnosis of malignant lymphoma. The most important, and often the most perplexing of these, is the canine venereal tumor. This lesion has been known for many decades and has been the subject of several investigations since the pioneer transmission experiments of Novinsky mentioned previously The disease is readily transmitted by coitus from infected to susceptible animals and can be experimentally induced by transfer of suspensions of cells from the tumors to normal canine genitalia The tumors appear on the penis or prepuce of the male and in the vagina and occasionally in the cervix and uterus of the female. Rather large fungoid masses are usually seen (Fig 13-1) which are friable, richly vascular, and occasionally necrotic Microscopic examination reveals the tumors to be made up of individually discrete cells with hyperchromatic

Rodents

Lymphocytic malignancies are infrequent but have been reported in the rat[21a] and, of course, have been studied a great deal in the mouse It is also rare as a spontaneous disease in hamsters and guinea pigs.

Monkeys

We have been unable to find a satisfactorily documented case of malignant lymphoma in a subhuman primate. It would be strange, indeed, however, if these species were not susceptible.

Cattle

Lymphocytic malignancies in bovine species are not infrequent; the disease is world-wide in distribution and enzootic outbreaks have been reported In general, the disease in cattle is not particularly well documented, although good descriptions of outbreaks and individual cases have appeared in the literature The disease is characterized by generalized invasion of lymph nodes by malignant lymphoid cells which frequently infiltrate the cardiac muscle [25] Acute forms of lymphocytic leukemia are sometimes seen in young calves Intense study of the disease in this species is definitely indicated and should be fruitful. It is hoped that certain studies now getting underway will yield information of value toward better understanding of the disease

Of interest is the recent study by Barnes[1] of the records of the United States Department of Agriculture relative to the incidence of lymphoma in cattle slaughtered for food in establishments under the meat inspection service of the Department It must be borne in mind that these records were not accumulated in order to study lymphoma, and the diagnoses were made for the most part upon gross examination of slaughtered animals in determining their fitness for human consumption Nevertheless, the figures accumulated are of interest in relation to the probable incidence of the disease in cattle According to these records, 230,170,948 cattle were slaughtered during the years 1944 through 1958, and lymphoma was recognized in 27,764 of these animals Between the years 1944 and 1953, malignant lymphoma was recorded at an annual rate per thousand slaughtered animals of from 9 0 to 11 5 During subsequent years, 1954 through 1958, this rate rose steadily to reach 18 0 per thousand If these records reflect the true status of the disease in cattle in this country, the incidence has been steadily increasing since 1953, and the 1958 rate is twice that of 1944 Research is definitely needed to evaluate this bovine disease by all means possible, to determine if this apparent increased incidence is real, and, if possible, to learn its causes



Fig 13-3 Canine venereal tumor, extragenital lesion The dermis is intensely infiltrated with cells which elevate the epidermis and invade the subcutis (× 8) (AFIP 710474)

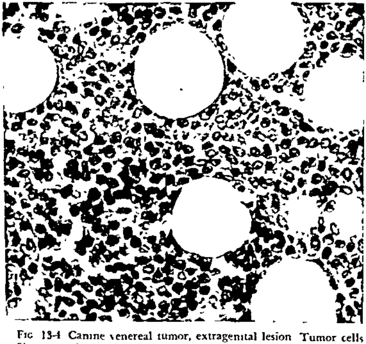


Fig 13-4 Canine venereal tumor, extragenital lesion Tumor cells *infiltrating subcutaneous fat (× 440) (AFIP 710474)*

spherical nuclei (Fig. 13-2). These cells are densely packed, often in mitosis, and infiltrate the tissues underlying the epithelium The resemblance of these cells to malignant lymphoid cells suggested one of its synonymous names, transmissible lymphosarcoma.

The origin and nature of the cells are still a matter of controversy Bloom, Paff, and Noback[4] consider them to be mature end cells of reticuloendothelial origin; Mulligan[21] relates them to histiocytes; Jackson[17] and DeMonbreun and Goodpasture[5] feels that their origin is uncertain but that they are



Fig 13 2 Canine venereal tumor Individually discrete cells infiltrating dermis (× 440) (AFIP 710474)

most closely related to the lymphocytic series In addition to being transmissible, this lesion is generally self-limiting, is confined to the genital system, and confers immunity to further attacks However, in a few animals metastases and extragenital lesions occur which may or may not be related

The extragenital lesion which simulates malignant lymphoma of the skin is histologically indistinguishable from the venereal tumor but is not identical in all clinical aspects This lesion appears rapidly in young dogs, usually as a single lesion which displaces the dermis and elevates the epidermis The lesions are circumscribed, usually from 2 to 5 cm in diameter, and are often ulcerated due to licking or scratching by the afflicted animal Histologically they are made up of individual round cells which infiltrate



FIG 13 3 Canine venereal tumor, extragenital lesion The dermis is intensely infiltrated with cells which elevate the epidermis and invade the subcutis (× 8) (AFIP 710474)

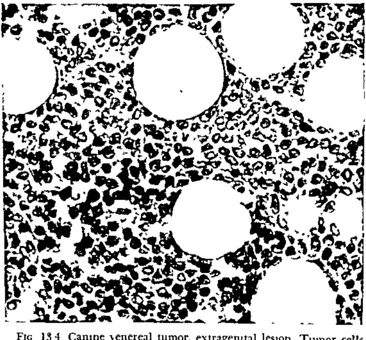


FIG 13 4 Canine venereal tumor, extragenital lesion Tumor cells infiltrating subcutaneous fat (× 440) (AFIP 710474)

the dermis (Fig 13-3), isolate the collagen bundles, and extend into the subcutis to grow between the fat cells (Fig. 13-4). Mitoses are common. In many respects, therefore, this lesion simulates a locally invasive, malignant neoplasm, but it actually is benign, readily cured by simple, even incomplete excision, and is often self-limiting Of some help to the pathologist encountering this lesion is the knowledge that malignant lymphoma in the

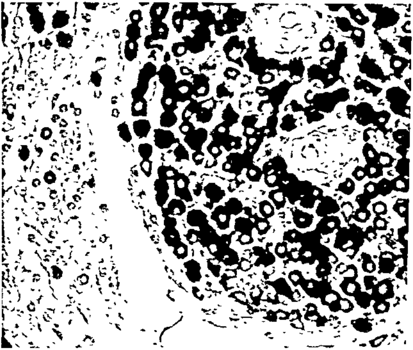


FIG. 13-5 Canine mastocytoma Neoplastic mast cells invade the dermis (× 375) (AFIP 186765)

dog rarely involves the skin, and when it does, other organs are usually recognizably affected

The canine mastocytoma may present a problem in differential diagnosis unless the pathologist is familiar with its characteristics This tumor involves the dermis and sometimes the subcutis It is made up of mast cells of varying degrees of differentiation and is mixed with eosinophils and occasionally more primitive myeloid cells The metachromatic granules in the cytoplasm of the mast cells are of definitive value in distguishing these cells (Fig 13-5)

The term "malignant lymphoma" as used by Gall and Mallory[12] most clearly encompasses the histologic features of the various forms of lympho-

cytic malignancies in the dog (as well as in most other species) Bloom and Meyer[3] using sections and imprints have classified twenty canine cases into *lymphoblastic, lymphosarcoma cell, lymphocytic, and mixed cell types.* We have seen a few cases which could be related histologically to the giant follicular lymphoblastoma of man. A few canine cases simulating Hodgkin's disease of man have also been reported.[20] At this time no clear-cut correlation *is evident between the various histologic or cytologic manifestations* and the biologic course of the disease.



FIG 13 6 Canine malignant lymphoma, ventral aspect of mandibular region with skin reflected All lymph nodes are greatly enlarged A male mongrel terrier dog, 12 years of age (Courtesy of Angell Memorial Animal Hospital)

The gross lesions in the dog are of interest, particularly in comparison with those of the cat and other species As in all species, it is often difficult, if not impossible, to determine the "primary" site of the lesions. More often than not it appears that several organs may be invaded by malignant lymphoid cells almost simultaneously This generalized manifestation most frequently affects the lymph nodes In some cases one organ may be particularly affected and may be greatly enlarged before other organs are seriously invaded This results in a lesion which is foremost when the

the dermis (Fig. 13-3), isolate the collagen bundles, and extend into the *subcutis to grow between the fat cells (Fig. 13-4). Mitoses* are common. In many respects, therefore, this lesion simulates a locally invasive, malignant neoplasm, but it actually is benign, readily cured by simple, even incomplete excision, and is often self-limiting. Of some help to the pathologist encountering this lesion is the knowledge that malignant lymphoma in the

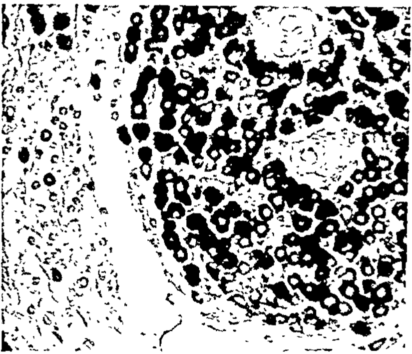


FIG. 13-5 Canine mastocytoma. Neoplastic mast cells invade the dermis and are often arranged concentrically around hyaline, thick-walled blood vessels. The metachromatic granules in this cytoplasm of the mast cells differentiate the cells from the lymphocytic series. Giemsa's stain (× 375) (AFIP 186765.)

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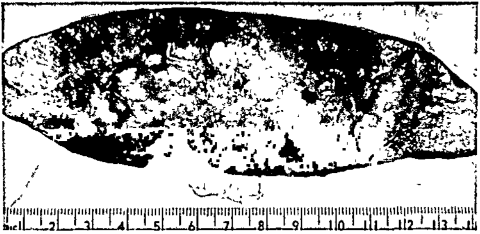


FIG. 13.8 Canine malignant lymphoma. Multiple masses of lymphoma and large sharply demarcated infarcts in the spleen of a 5 year old male German shepherd dog. (Courtesy of Angell Memorial Animal Hospital.)

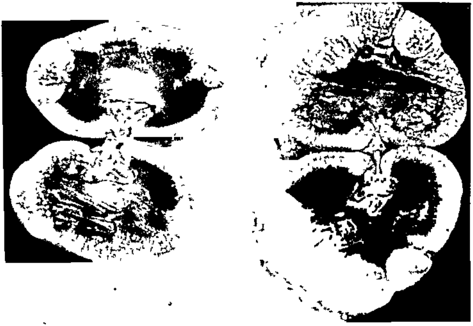


FIG. 13.9 Feline malignant lymphoma. Large tumor masses in the renal cortex of a 10 year old castrated male cat. (Courtesy of Angell Memorial Animal Hospital.)

animal is first presented for diagnosis or is first observed to be ill. This "presenting" lesion affords a means of making interesting comparisons between species and may determine the significant signs and course of the disease

Upon reviewing 147 cases of canine malignant lymphoma from the files of the Angell Memorial Animal Hospital, it was found that in 65 per cent of the cases, the presenting lesion was generalized lymphadenopathy (Fig



Fig 13-7 Canine malignant lymphoma A large mass in the wall of the ileum of a 6-year-old male German shepherd dog (Courtesy of Angell Memorial Animal Hospital)

13-6) In 12 per cent the thoracic lymph nodes were initially involved, resulting in respiratory difficulty *Single lesions in the small intestine (Fig 13-7) in* 5 per cent of the cases resulted in obstruction or stasis of the intestinal tract Large masses in the spleen (Fig 13-8) were the initial lesion in 2 per cent of the cases, although early infiltration of lymph nodes was usually present In only 2 per cent was a leukemic blood picture observed, and this was usually seen only in the terminal stages of the disease Renal lesions were outstanding in only 2 per cent of the canine cases

The incidence of malignant lymphoma among dogs admitted to the Angell Memorial Animal Hospital increased slowly from 0 4 in 1948 to 1 54 per 1,000 admissions in 1953 During the next five years, 1954 through 1958,

the incidence has risen slightly from 2.11 to 2.5 per 1,000 admissions. (About 8,000 dogs are admitted each year to this animal hospital.) It is probable that this apparent increase in frequency of occurrence is not based upon an actual higher incidence but rather upon the increased interest in the disease and its more frequent recognition. (Reference is made to Fig. 13-12.)

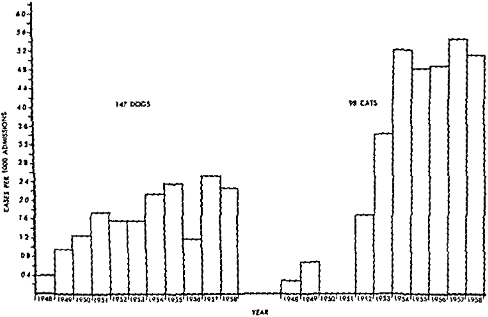


Fig. 13-12 Annual rate per 1000 admissions of malignant lymphoma in 147 dogs and 98 cats

The age at which dogs may be affected with malignant lymphoma is portrayed graphically in Figure 13-13 It will be seen that the disease occurs in dogs from the first through the sixteenth year of age The disease is seen most frequently in dogs between the third and ninth years of age Dogs in *this age group probably constitute the greatest segment of the canine population.*

Cats

The cat presents some interesting contrasts to the dog not only in temperament but in the way it is affected with malignant lymphoma The distribution of the gross lesions differs particularly between these two species. The *presenting lesion in the largest group (20 per cent) of the feline cases is*



Fig. 13-10 Feline malignant lymphoma Tumor masses filling anterior mediastinum (Courtesy of Angell Memorial Animal Hospital.)



Fig 13 11 Feline malignant lymphoma Neoplastic enlargement of mesenteric lymph node The intestinal wall usually is invaded when the adjacent lymph nodes are affected (Courtesy of Angell Memorial Animal Hospital)

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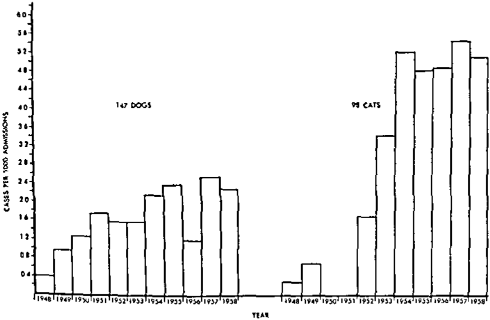


FIG 13 12 Annual rate per 1000 admissions of malignant lymphoma in 147 dogs and 98 cats

The age at which dogs may be affected with malignant lymphoma is portrayed graphically in Figure 13-13 It will be seen that the disease occurs in dogs from the first through the sixteenth year of age The disease is seen most frequently in dogs between the third and ninth years of age Dogs in this age group probably constitute the greatest segment of the canine population.

CATS

The cat presents some interesting contrasts to the dog not only in temperament but in the way it is affected with malignant lymphoma The distribution of the gross lesions differs particularly between these two species. The presenting lesion in the largest group (20 per cent) of the feline cases is

located in the kidneys. These lesions (Fig. 13-9) often result in death due to renal failure before any significant gross lesions are evident elsewhere in the body. In a nearly equal group (18 per cent) the anterior mediastinum (and presumably thymus) is filled with tumor (Fig. 13-10), thereby making the initial signs referable to respiratory difficulty. This is a feature that also occurs in certain strains of mice affected with malignant lymphoma

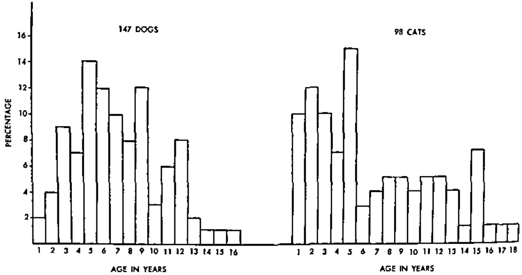


FIG. 13-13 Age distribution of malignant lymphoma in 147 dogs and 98 cats

The liver is primarily affected in 17 per cent of the feline cases, and the presenting signs are referable to liver failure This infiltration may be diffuse or nodular Single nodules of malignant lymphoma in the small intestine or mesenteric lymph nodes are found in about 15 per cent of the cases (Fig 13-11) Palpable abdominal masses and intestinal obstruction are therefore fairly frequent clinical findings Leukemic blood picture is encountered in about 10 per cent of the cases in cats

The incidence of malignant lymphoma in cats appears to be nearly twice that of dogs (Fig 13-12) The average rate per 1,000 admissions during the years 1954 through 1958 was about 5 5 The records prior to these years probably are incomplete The age of affected cats ranges from a few months to seventeen years (Fig 13-13) A significantly greater number of young animals of this species are affected in comparison with the dog

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CHAPTER 14

EXPERIMENTAL INDUCTION OF LYMPHOCYTIC MALIGNANCIES

STEVEN O. SCHWARTZ

Our interest in human leukemia compelled the search for a tool to enable the investigation of the etiologic factors responsible for the develeopment of the disease. Because of similarities between lymphatic leukemia in the mouse and human leukemias, we undertook a series of experiments to test these similarities in the two species. We have learned that antigenic similarities do exist regarding the white cells and red cells and that the changes in the blood, the marrow, lymph nodes, spleen, liver, lungs, kidneys, and genital organs resemble one another

Experiments in which cell-free filtrates were prepared from the brain of leukemic mice soon convinced us that leukemia could be induced by means of a cell-free agent whose characteristics were those of a virus.

The agent was found to be self-perpetuating in passages through mice Titration of the agent revealed a progressive fall of activity with almost no ability to induce leukemia in a dilution of 10^5 However, in serial passages through animals of the same species, the cell-free filtrate can be diluted virtually to infinity In our own experiments the dilutions have been carried out as high as 10^{44} with excellent retention of leukemia-inducing ability

One of the intriguing questions in this field is whether leukemia primarily represents a metamorphosis of cells, which would equate it with the carcinomas, or whether it is primarily a reactive hyperplasia, which later becomes autonomous and irreversible If leukemia represents a metamorphosis of cells, one must assume a unicentric origin from a focus where cells have become "malignant," where they multiply, and from which they metastasize. If, on the other hand, leukemia represents a reactive hyper-

21 MULLIGAN, R. M. Neoplastic diseases of dogs II Mast cell sarcoma, lymphosarcoma, histiocytoma. *Arch Path. 46:*477-492, 1948.

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By seventy-two hours the changes described are more definite. The cellular infiltration of the fat tissue around the genital organs and the kidneys is almost entirely made up of the mononuclear cells with only an occasional polymorphonuclear leukocyte. The periadrenal fat tissue is similarly involved.

By the fourth day the changes are unmistakable. In those areas in which small collections of tumor cells were seen earlier, tumor cell infiltration is heavier. In addition the tissues around the mediastinal and mesenteric nodes also show beginning infiltration with tumor cells, although the architecture of the lymph nodes remains preserved.

On the fifth and sixth days changes are progressing, an occasional lymph node shows specific involvement. By the seventh day the mesenteric and mediastinal lymph nodes show certain involvement, and the inguinal lymph nodes show early changes. By the eighth day the lymph node involvement becomes generalized By the ninth day complete dissemination has occurred; lymphoblastoma is fully developed.

The progression of changes is essentially the same in all animals injected with leukemic material. Significant differences could not be demonstrated between the development of leukemia in those animals injected intracerebrally or intraperitoneally with leukemic cell-free filtrates and those animals injected with tumor cell suspensions

The very early lymphoblastomatous transformation in the fat tissues surrounding different organs is striking. This occurs long before significant changes can be demonstrated in such lymphoid tissues as lymph nodes, thymus, and spleen.

A contiguous involvement from the site of injection seems unlikely because the progression of changes in the animals injected with tumor cell suspensions intraperitoneally does not differ significantly from the progression of changes seen in animals injected intracerebrally with cell-free filtrates This progression offers further evidence of a muticentric leukemic transformation. The leukemic transformation is believed to be due in both instances to the proliferation of a virus In the case of cell-free filtrates, the virus is present in free form; in the case of cell inoculation, the virus *is present in the cells* from which it is liberated

CONCLUSION

It may be concluded that leukemic cell-free filtrates elicit a lymphoid proliferation. This lymphoid proliferation has its genesis in the mesenchymal cells. The proliferation eventually leads to an irreversible overgrowth Crowding out and replacement of normal tissues take place There is evidence that the leukemia represents a multicentric response rather than a local growth which spreads by virtue of metastases

plasia, then we may expect a multicentric origin, secondary to a mobilization of primitive mesenchymal cells.

EXPERIMENTS ON THE GENESIS OF LEUKEMIA

A series of experiments was undertaken to study the genesis of leukemia. One group of animals received intracerebral injections and one group, intraperitoneal injections of leukemic cell-free filtrate from the brain, a third group was injected with cell-free filtrate of brain from nonleukemic mice, and a fourth group was injected intraperitoneally with tumor cells suspension. It was known that lymphoblastoma would develop in the animals a week or two after inoculation; a number of animals from each group were sacrificed each day. The tissues were studied as unknowns. Animals were considered positive only when the diagnosis of lymphoblastoma could be made unequivocally

We were particularly interested in ascertaining when and where the earliest lymphomatous changes take place We wished to know also whether the leukemic transformation occurred in different areas sequentially or simultaneously. We wanted to learn, further, whether the way the leukemia develops differs when cell-free filtrates or tumor cells are used for purposes of induction, or when intracerebral or intraperitoneal routes are used for inoculation.

Both C3H and Swiss mice were used. One tenth of a cubic centimeter of the cell-free filtrates was used for the intracerebral and 0 5 cc, for the intraperitoneal inoculation In the tumor cell suspension experiments, 0 5 cc of a tumor cell suspension containing approximately 10^6 cells per cubic centimeter was used By the eighth day all the injected mice presented gross evidence of leukemia

The fully manifest lymphoblastoma shows a complete obliteration and replacement of lymph node architecture, tumor cell invasion of the perinodal tissues, invasion of the salivary glands, and heavy infiltration of the liver, kidneys, perirenal tissue, and genital organs The skeletal muscles, spleen, marrow, and central nervous system are inconstantly involved. The progression of morphologic changes as observed at twenty-four hour intervals was as follows

In twenty-four hours there were no specific changes Some of the lymph nodes show a nonspecific lymphadenitis

In forty eight hours the earliest changes are seen in the fat tissue of the mesentery, around the kidneys, and around the genital organs, where mononuclear cells appear which resemble large lymphoblasts These cells are intermingled with polymorphonuclear leukocytes The lymph nodes show lymphadenitis Such changes are not seen in the animals inoculated with nonleukemic brain filtrates

CHAPTER 15

THE CHANGING PATTERN OF LYMPHOCYTIC MALIGNANCIES

R. PHILIP CUSTER

Doctor Schwartz' chapter (Chapter 14) is a most beautiful and convincing demonstration of cellular multipotentials Not only does he show the development of leukemic cells from indifferent reticular elements but from differentiated cells as well, notably those of the renal capsule, which we generally assume to be relatively inert fibrocytes After Doctor Schwartz' presentation I feel much better about my habitual use of the term "leukemic metaplasia" in place of the conventional "leukemic infiltration" Leukemic cells unquestionably can and do colonize from the peripheral blood, as well as infiltrate, but I have long maintained that their interstitial growth can likewise be autochthonous from preexisting elements, probably reticulo-endothelial for the most part

In one of my earlier papers,[1] the following statement was made: "The concept of the cell as a static unit must be discarded" This was based on experience with a large number of cases of leukemia and lymphoma in which tissue was sampled at various times during the course of the disease, and autopsy material generally obtained In collaboration with Dr William Bernhard, the results of our first analysis were published in 1948,[2] conforming substantially to the flow diagram shown in Figure 15-1. In over 35 per cent of cases there was a significant change in the histologic character of the malignant growth, an incidence confirmed recently by Doctor Edward Gall[3]

While the occurrence of such transitions has been commoner among the lymphomatous disorders, they are by no means confined to this category. Changes in the myeloproliferative group, coupled with malignant plasmocytic disease, are shown in Figure 15-2, the latter merely representing geo-

Leukemia represents in the beginning not a wild, autonomous, purposeless proliferation but an unsuccessful biologic response, a response that is originally defensive in nature. Why this process eventually becomes autonomous and lethal remains to be answered.

EDITOR'S NOTE: For documentation the reader is referred to the recent review article Schwartz, S. O., and Schoolman, H. M. The etiology of leukemia. The status of the virus as causative agent—A review. *Blood 14*: 279, 1959.

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FOLLICULAR TO LYMPHOCYTIC TO RETICULUM CELL LYMPHOMA

Case 2. This 62-year-old man gave the history of a mass developing rather rapidly in his right axilla fifteen years earlier. It reached the size of a golf ball and remained static until five weeks prior to admission. At that time he noted enlargement of all superficial nodes, associated with progressive

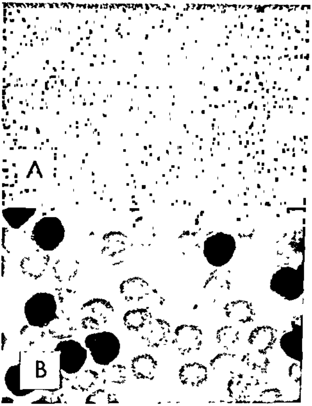


FIG 15-4 *Case 1* Axillary lymph node biopsy diagnosed as follicular lymphoma (*A*) was followed a year later by the development of chronic lymphocytic leukemia (*B*), the peripheral leukocyte count reaching 250 000 per cubic millimeter (all mature lymphocytes)

abdominal swelling The latter was identified as massive tumor growth, apparently a composite of nodal, splenic, and hepatic enlargement. Removal of the original axillary mass, now the size of a tennis ball, disclosed a malignant lymphoma of follicular type (Fig 15-5A). He responded well to chemotherapy, and subsequent local recurrences were treated by radiotherapy A biopsy about a year later showed complete loss of nodular pattern (Fig

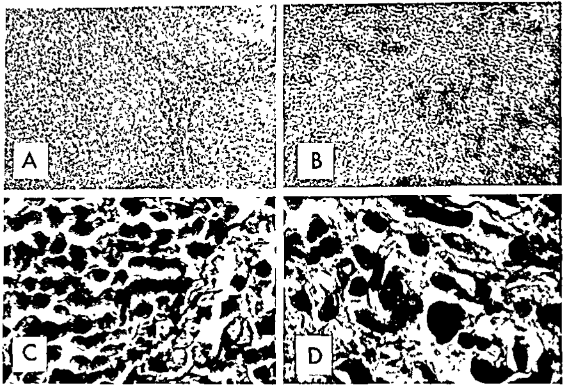


FIG 15 5. *Case 2* Original follicular lymphoma (*A*), present for 15 years, showed loss of nodular pattern a year later (*B*), with the tumor composed exclusively of small lymphocytes (*C*) At autopsy, 1½ years after admission, the cytologic pattern was of pleomorphic reticulum cell type (*D*).

FOLLICULAR TO LYMPHOCYTIC TO RETICULUM CELL LYMPHOMA

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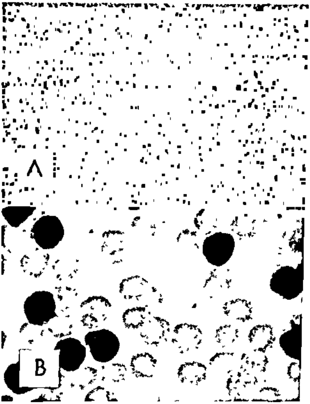


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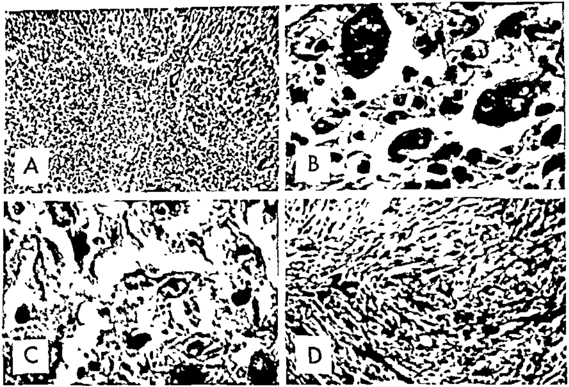


FIG 15 6 *Case 3* Original biopsy showing follicular lymphoma (*A*), treated by roentgen therapy, was followed by Hodgkin's lymphoma 9 years later which had the composite picture of granuloma (*B*), reticulum cell sarcoma (*C*) and fibrosarcoma (*D*).

15-5*B*), the growth now forming a diffuse sheet of small lymphocytes (Fig. 15-5*C*). Three and one-half years after his first admission there was major recurrence, and the tumor proved resistant to various therapeutic measures He developed panhematocytopenia and died of sepsis incident thereto The tumor found at autopsy was composed entirely of large, pleomorphic reticulum cells (Fig. 15-5*D*).

FOLLICULAR LYMPHOMA TO MULTIFACETED HODGKIN'S TYPE

Case 3. A white woman 49 years old was admitted to the Cardio-Thoracic Surgical Service for resection of a large mediastinal tumor which proved to be Hodgkin's lymphoma, some parts being typically granulomatous (Fig. 15-6*B*), others purely sarcomatous (Fig. 15-6*C*), while still others showed a fibrosarcomatous pattern (Fig. 15-6*D*) On being questioned later about an axillary scar, she recalled having had a node removed nine years previously, a course of x-ray therapy followed We were able to obtain a section of the biopsy which showed the follicular type of lymphoma (Fig 15-6*A*).

FOLLICULAR LYMPHOMA TO DESMOPLASTIC HODGKIN'S TYPE

Case 4 This was the fascinating case of a woman who, at the age of 51, developed bilateral supraclavicular tumors which remained relatively static for four years and then began to enlarge rapidly. A biopsy showed the tumor to be a follicular lymphoma (Fig 15-7*A*) which was controlled by chemotherapy for the next three years A large mass then appeared in the left lower quadrant of the abdomen and regressed after treatment with x-rays and alkylating agents Five months later she was readmitted with the complete syndrome of malignant hypertension, striking cardiac enlargement having occurred in the interim A Goldblatt kidney situation was suspected, treatment directed toward the abdominal tumor was resumed, and a brief respite was obtained The patient then became virtually moribund, and, mostly as a gesture, an additional 15 mg of triethylene melamine were administered To the amazement of everyone, symptoms were gradually relieved over the next three weeks, and she became ambulatory and comfortable during the subsequent two years Recurrence of malignant hypertension, this time unaffected by chemotherapy, led to her final admission, and she died as the result of renal failure complicated by staphylococcic pneumonia

The autopsy disclosed the left kidney encased by dense white tissue, its major blood vessels rendered stenotic thereby The right renal artery was likewise constricted, and both kidneys were the seat of pyelonephritis Sections of the tumor mass showed it to be composed chiefly of acellular collagenous tissue punctuated by virtually empty spaces (Fig 15-7*B*), one of

MYCOSIS FUNGOIDES TO LYMPHOCYTIC LYMPHOMA

Case 6. This patient was referred from the Dermatology Service in 1953. Biopsy of red, nodular skin lesions led to the diagnosis of mycosis fungoides (Fig. 15-9*A*), regression following treatment with triethylene melamine.

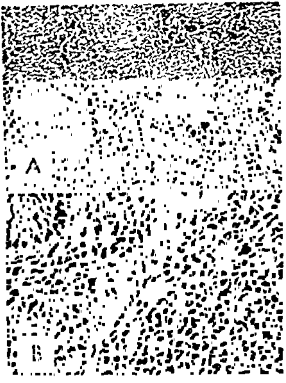


FIG. 15-8 *Case 5* Follicular lymphoma in mesocolic lymph nodes (*A*) with coexistent mixed lymphocytic and reticulum cell lymphoma in the wall of the adjacent colon (*B*)

Within a month the enlargement of axillary lymph nodes indicated systemic spread, and aspiration of iliac bone marrow confirmed this However, both bone marrow and lymph node biopsies were different from that of the skin, the malignant process in each being purely a well-differentiated lymphocytic growth (Fig 15 9*B*). Since then splenomegaly has been the notable manifestation of the lymphomatous process, occasionally coupled with nodal enlargement and both controlled by a minimum of radiotherapy or chemotherapy as indicated The patient remains clinically well and works steadily as a commercial photographer

which contained a few residual elements including a Reed-Sternberg cell (Fig. 15-7C) It is assumed that the cellular nests generally encountered in this variety of lymphoma were depleted by the chemotherapeutic agent.

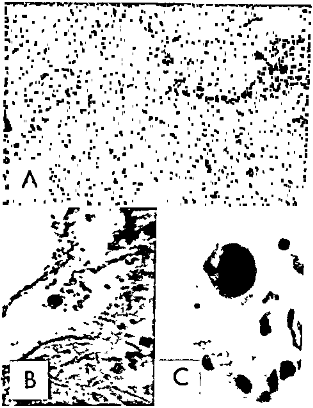


FIG 15-7 *Case 4* Biopsy of follicular lymphoma (*A*) controlled by irradiation and chemotherapy for 5 years Autopsy disclosed a desmoplastic type of Hodgkin's lymphoma, the tumor being composed almost entirely of acellular collagenous tissue (*B*, lower right), with scattered spaces containing a few cellular elements, including a Reed-Sternberg form (*C*).

Coexistence of Follicular and Mixed Lymphocytic and Reticulum Cell Lymphoma

Case 5 This was the case of a middle-aged man, a segment of whose colon was removed along with a group of enlarged mesocolic lymph nodes because of partial obstruction Sections of the latter showed the nodular type of lymphoma (Fig 15-8*A*), while the tumor in the intestinal wall was made up of an admixture of lymphocytes and reticulum cells (Fig. 15-8*B*).

FOLLICULAR LYMPHOMA TO HODGKIN'S GRANULOMA, TERMINATING AS ACUTE GRANULOCYTIC LEUKEMIA

Case 8. This is one of our rare links between lymphomatous and myeloproliferative disorders. The patient, a 76-year-old woman, noted a lump in

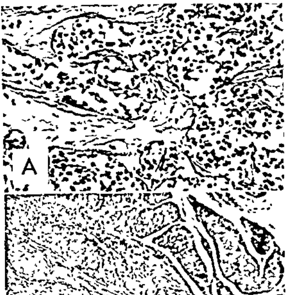


FIG 15 10 *Case 7* A rapidly growing retroperitoneal lymphangiosarcoma (*A*) regressed following radiotherapy, but was followed by follicular lymphoma 18 months later (*B*) At the time of the latter biopsy the nodular pattern was fading and the growth was assuming a reticulum cell form

her neck in June, 1951, it regressed spontaneously and recurred three months later, the regrowth being rapid and multinodular. The biopsy viewed at low magnification was folliculoid (Fig 15-11*A*), but the cellular components were typical of Hodgkin's granuloma, complete with Reed-Sternberg forms and eosinophils as part of the multiplicity of cell types (Fig 15-11*B*) The initial growth was checked by radiotherapy, but within the year generalized dissemination required the use of alkylating agents.

LYMPHANGIOSARCOMA FOLLOWED BY FOLLICULAR LYMPHOMA MERGING WITH RETICULUM CELL SARCOMA

Case 7. A rapidly growing lower abdominal tumor was investigated by laparotomy and found to be retroperitoneal in location, biopsy identifying

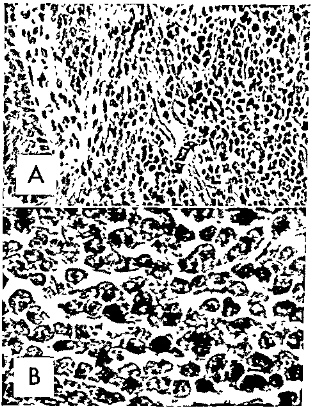


FIG 15-9. *Case 6* Skin biopsy of mycosis fungoides (*A*), followed a few months after treatment by well-differentiated lymphocytic lymphoma (*B*) in lymph nodes and bone marrow

it as a lymphangiosarcoma (Fig 15-10*A*) Complete regression followed radiotherapy Eighteen months later the patient's cervical lymph nodes enlarged, one of these was removed and found to be the seat of follicular lymphoma (Fig 15-10*B*) The pseudofollicles were already in the stage of coalescence, the growth assuming a reticulum cell sarcomatous pattern (This case was supplied by courtesy of Dr David Rosenbaum, Veterans Administration Hospital, Indianapolis, Indiana)

Nonetheless, she remained active and comfortable for another year, toward the end of which the blood studies during her periodic check-ups showed a steady increase in circulating myeloblasts. In September, 1953, she developed herpes zoster, requiring readmission to the hospital, and close observation of the blood picture disclosed progressive anemia and thrombocytopenia with increasing myeloblasts. Sections of aspirated bone marrow units revealed virtually complete displacement of normal hematopoietic tissue by a homogeneous sheet of myeloblasts (Fig 15-11C). A remission lasting two months was obtained by combined antimetabolite and corticosteroid therapy, but the patient died in November, 1953. The autopsy findings were purely those of acute granulocytic leukemia, no vestige of the original lymphoma being present.

SUMMARY

Through the study of many such cases, it becomes apparent that the distinctive cytologic patterns in the lymphoma-leukemia complex do not denote separate neoplastic entities. They appear to be varied expressions of a single malignant process stemming from cellular derivatives of the embryonal mesenchyme. Thus, the neoplastic proliferation may remain pure as to cell type from the outset, may become composite, or may alter completely during the course of the disease.

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4 Lawrence, J H, and Rosenthal, R L Multiple myeloma associated with polycythemia. Report of four cases *Am J Med Sc 218* 149 154, 1949

The illustrations in this chapter were reproduced from the original color photomicrographs much of the cytologic detail was lost in the process



FIG. 15.11. *Case 8.* A cervical lymph node biopsy viewed at low magnification showed vestiges of follicular lymphoma (*A*). Closer inspection (*B*) disclosed a multiplicity of cell types, including eosinophils and Reed-Sternberg cells typical of Hodgkin's granuloma. After therapeutic control of the tumor for two years, the patient developed acute granulocytic leukemia, aspirated fragments of bone marrow showing complete replacement of hematopoietic tissue by diffuse growth of myeloblasts (*C*).

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CHAPTER 16

HISTOCHEMISTRY OF LYMPHOCYTIC TISSUE IN THE MALIGNANT LYMPHOMAS

HERBERT BRAUNSTEIN

This chapter concerns itself with the histochemistry of enzymes in lymphoid tissue and malignant lymphoma. Most of the material to be presented was studied in our own laboratory in conjunction with Dr. David G. Freiman and Dr Edward A Gall

The development of histochemical techniques for the demonstration of enzymes in situ has permitted the study of tissue sections to determine the intracellular sites of activity Most methods for demonstrating enzymes depend upon the use of artificial substrates, with the aim being the formation of an insoluble precipitate in the exact area of activity For purposes of this presentation, I have divided the enzymes we have studied histochemically into two groups, hydrolytic and metabolic

We have studied the first group more intensively since these enzymes may usually be demonstrated following the use of more conventional methods of tissue processing This group, in general, tends to be confined to specific cells rather than generally distributed However, little or no knowledge, with rare exceptions, is available concerning their intracellular functions The second group, metabolic enzymes, tends to be more widely distributed, especially those concerned with oxidative activity These usually require the use of fresh, unfixed tissue for their demonstration We know generally what their metabolic functions are

The present study will indicate the distribution of enzymes in malignant lymphoma and will compare this with the activity of the normal lymphoid elements Two possible purposes may be served by this survey

(1) identification of specific cells by their enzyme activity and (2) a study of the possible differences in metabolism of neoplastic cells as contrasted with their normal counterparts. The currently popular concept of enzyme adaptation and observations indicating an apparent tendency toward enzyme deletion in malignant neoplasms are evidences of the potential importance of the latter.[1]

HYDROLYTIC ENZYMES IN NORMAL LYMPHOID CELLS

Table 16-1 summarizes the hydrolytic ezyme activity of the normal lymphoid cells. As pathologists our belief is that lymphoid elements represent distinct morphologic units and that differences in morphology will probably be accompanied by functional differences.[2]

Lymphocytes. The lymphocyte, the predominant nodal cell, manifests only probable acid phosphatase activity but no esterase, alkaline phosphatase, 5 nucleotidase, phosphamidase,[3] or leucine aminopeptidase. We have not studied β-glucuronidase in humans. Studies of blood smears and lymph node imprints have confirmed the presence of slight acid phosphatase activity in 50 to 90 per cent of normal lymphocytes.

Histiocytes. Histiocytes in all locations in the lymph node manifest strong esterase, acid phosphatase, and phosphamidase activity.[3] Slight leucine aminopeptidase activity is also present,[6] while studies by others have revealed β-glucuronidase as well.[5] Blood monocytes, like histiocytes, contain considerable acid phosphatase and esterase, but we have not studied enzymes other than these and alkaline phosphatase. Germinal center cells reveal moderate 5-nucleotidase activity[7] but are otherwise unreactive, while capillary endothelium is strongly positive for alkaline phosphatase only.

Lymph Node Follicles When the normal lymph node follicle was stained to demonstrate nonspecific esterase, the isolated histiocytes were positive (black) while germinal center cells were negative. Study of the same follicle in a preparation demonstrating acid phosphatase activity revealed that activity restricted to histiocytes as manifested by black precipitate. When stained to demonstrate 5-nucleotidase, the cells of the germinal centers were positive (black), while the areas within them, where histiocytes are located, were devoid of activity

Other Lymphoid Elements. When a lymph node showing the typical pattern of sinus hyperplasia was stained to demonstrate esterase, the histiocytes lining the peripheral lymph sinus stained strongly In a similar section stained to demonstrate acid phosphatase,[7] the sinus histiocytes contained considerable bright-red precipitate Leucine aminopeptidase activity in histiocytes was shown by the red precipitate in sinus lining cells In sections of spleen stained to show acid phosphatase, the red pulp manifested intense

Table 16-1. Hydrolytic Enzymes in Normal Lymphoid Elements

Cell	*Esterase*	*Alkaline phosphatase*	*Acid phosphatase*	*5-Nucleotidase*	*Phosphamidase*	*β Glucuronidase*	*Leucine aminopeptidase*
Lymphocyte	0	0	Node ?+ Blood many +	0	0	+ (Rat) [4]	0
Histiocyte	4+ node Monocyte +	0	3–4+ node Monocyte +	0	2+ node	+ [5]	1+ node
Germinal center	0	0	0	2+	0	0 (Rat) [4]	0
Endothelium	0	4+	0	0	0	—	0

KEY 0 = a negative reaction, — = data unavailable, ? = uncertainty as to any reaction.

red precipitate in the sinus histiocytes, while the follicles were pale due to the lesser activity of the lymphocytes. In imprints of a lymph node stained to demonstrate acid phosphatase, the large cells with the intense staining reaction were histiocytes while the lymphocytes contained only minute, scattered granules With previously utilized techniques, it was not possible to demonstrate acid phosphatase in lymphocytes. In normal blood smears stained to demonstrate esterase, the monocyte was strongly positive in contrast with the lack of staining in neutrophils Similar smears demonstrated strong acid phosphatase activity in the blood monocyte.

HYDROLYTIC ENZYMES IN LYMPHOID DISEASES

Table 16-2 illustrates the distribution of the same enzymes in lymphoid diseases. Note that the epithelioid cells of granulomas manifest similar activity to histiocytes[8] but with increased leucine aminopeptidase activity. Proliferating fibroblasts in areas of inflammation and in Hodgkin's disease are strongly positive for acid and alkaline phosphatases and leucine aminopeptidase. The cells of histiocytic lymphoma and monocytic leukemia are qualitatively identical with their normal counterparts[2, 8] Indeed, stem cell lymphoma may be distinguished from the histiocytic type on the basis of its lack of enzyme activity Sternberg cells and tumor histiocytes of Hodgkin's disease also manifests similar activity to that of normal histiocytes.[2, 8] One observation of possible significance is that while acid phosphatase and leucine aminopeptidase activities are high in these neoplastic histiocytes, many cells seem to manifest diminished esterase activity in contrast to normal histiocytes Lymphocytic lymphoma cells are inactive with the probable exception of acid phosphatase In the blood, leukemic lymphocytes manifest acid phosphatase activity virtually universally In one case of follicular lymphoma, activity of lymphocytes resembled that seen in the diffuse form.

When a type of lymphoma demonstrating the pattern of total replacement by proliferating histiocytes characteristic of histiocytic lymphoma was stained to demonstrate esterase, most of the cells were stained Many appeared to manifest lesser degrees of activity than normal. This is significant in view of the reported diminution or absence of this enzyme and other catabolic enzymes said to be characteristic of malignant tumors in general.[1] The acid phosphatase stain in histiocytic lymphoma showed enormous activity, so strong that individual cells could not be distinguished The activity did not appear to be diminished In a blood smear from a case of monocytic leukemia, leukemic monocytes manifested esterase activity. In a similar instance the monocytes showed strong acid phosphatase activity Neutrophils in the smear likewise contained much acid phosphatase Study of sectioned lymph node material showing typical Hodgkin's lymphoma revealed esterase

Table 16·2 Hydrolytic Enzymes in Lymphoid Diseases

Cell	*Esterase*	*Alkaline phosphatase*	*Acid phosphatase*	*5-Nucleotidase*	*Phosphamidase*	*β Glucuronidase*	*Leucine aminopeptidase*
Proliferating fibroblast	0	3+	Probably +	0	0	—	3+
Epithelioid cells	1+	0	4+	0	1–2+	—	4+
Histiocytic lymphoma	Mostly + Many normal	0	3–4+	0	1–2+	—	—
Monocytic leukemia	Mostly + Many normal	0	3–4+	—	—	—	—
Sternberg cells and Hodgkin's histiocytes	Mostly + Many normal	0	1+	0	1–2+	—	+
Stem cell lymphoma	0	0	0	0	0	—	0
Lymphocytic lymphoma	0	0	?+	0	0	Said to be 0	0
Lymphocytic leukemia	Probably 0	0	Mostly +	—	—	—	—
Follicular lymphoma	0	0	?+	0	0	—	0

KEY 0 = a negative reaction, — = data unavailable, ? = uncertainty as to any reaction

activity in tumor histiocytes and Sternberg cells Again, activity, although definitely present, frequently appeared to be diminished as compared with normal. The same case demonstrated acid phosphatase in Sternberg cells and histiocytes Leucine aminopeptidase was similarly shown in the same instance.

Examination of the typical proliferation of apparently mature lymphocytes in lymphocytic lymphoma in sections stained for esterase manifested the crowding out of the positively stained histiocytes by the proliferating lymphocytes. However, in several other instances, especially those from chronic lymphocytic leukemia, the lymph node architecture remained almost intact. Acid phosphatase activity (represented by minute, red cytoplasmic granules) was typically seen in the lymphocytes of blood smears of chronic lymphocytic leukemia Sections illustrative of the typical nodular morphology seen in follicular lymphoma were stained to demonstrate esterase Histiocytes were crowded out by the lymphocytes in the follicles but were apparent in the compressed adjacent nodal tissue.

METABOLIC ENZYMES IN LYMPHOID ELEMENTS

Only preliminary work has been begun on the group of enzymes depicted in Table 16-3, largely these concerned with intermediate metabolism. These include succinic dehydrogenase,[9] monoamine oxidase,[10] DPN and TPN diaphorases,[9] adenosine triphosphatase,[11] glucose 6-phosphatase,[12] and phosphorylase [13, 14] Note that lymphoid elements appear to be unreactive with the last three. In general, lymphocytes seem to possess strong succinic dehydrogenase and DPN diaphorase,[1] especially in the spleen, while TPN diaphorase and monoamine oxidase activities are weak. Activity of all four enzymes is greater in histiocytes within lymph nodes but is probably less in the spleen. More active cells, such as epithelioid cells, appear to have heightened activity of this group of enzymes. Thus far, in the only two cases of lymphoma studied, the enzymes appeared to be present in the cells in normal or slightly subnormal concentrations No definitive statements are as yet permissible on the basis of our data.

CONCLUSION

We have been able to identify lymphoid cells with reasonable certainty using enzymatic techniques Perhaps a wider use of these methods will take some of the confusion out of the nomenclature of malignant lymphomas It is also hoped that we shall increase our knowledge concerning metabolic derangements of the neoplastic cells in these conditions.

Table 16-3. Metabolic Enzymes in Lymphoid Elements

Cell	*Adenosine triphos phatase*	*Succinic dehydro genase*	*Monoamine oxidase*	*DPN diaphorase*	*TPN diaphorase*	*Phosphory-lase*	*Glucose 6-phosphatase*
Lymphocyte	0	Spleen, strong Node, moderate	0 to weak	Spleen, strong Node, moderate	Weak +	0	0
Histiocyte	0	Node > spleen	Lympho-cyte	Probably lympho-cyte > node	Lymphocyte	0	0
Germinal center	0	Spleen strong	Probably 0	Spleen, strong	?	0	0
Epithelioid cells	0	Very strong	+	Strong +	—	—	—
Lymphocytic lymphoma	0	Weak +	—	Moderate to strong +	—	—	—
Follicular lymphoma	0	Weak +	0 to weak	Moderate to strong +	Weak +	—	—

Key 0 = a negative reaction, — = data unavailable; ? = uncertainty as to any reaction

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CHAPTER 17

HISTOPATHOLOGIC BASIS FOR EVALUATION OF PROGNOSIS AND RESPONSE TO THERAPY OF THE LYMPHOCYTIC LYMPHOMAS

MATTHEW BLOCK

The microscopic criteria for the recognition of the various types of lymphomas have been fairly well standardized for many years. More recently our attention has been turned to correlation of histopathology with response to therapy and prognosis of each of the diseases comprising the broad group of lymphomas It is the purpose of this chapter to review this latter aspect and add my own experience based on histopathology *and* clinical care of the patient

PRELIMINARY CONSIDERATIONS

Technique

The histopathologic approach to malignant lymphomas is based upon an analysis of architectural pattern and of cytology It is therefore logical that a histologic technique be used which is adequate for both of these goals Unfortunately, over the course of years, preparation of tissue sections has been relegated to the technician Lumb[17] is the only author in recent years who has been properly concerned with histopathologic technique Others have failed to consider the subject or did so only briefly The delicacy of the hematopoietic tissues has been overlooked, and as a result specimens have been handled without due regard for the specific techniques needed to demonstrate adequately cytologic detail It is illogical that a microscopist, who examines blood cells only after they have been stained by one of the

Romanovsky mixtures, should be content to study the same cells in tissue sections after Formalin fixation and hematoxylin and eosin staining.

The following steps should be carried out so that sections adequate for cytologic analysis can be obtained.[8] Tissue should be sliced into pieces 2 or 3 mm. in thickness and immediately immersed into fixative for no longer than 2 or 3 hours. Refrigeration of the tissues overnight or even for a few hours[37] is not a substitute for immediate fixation Embedding must be done carefully to avoid shrinkage. If nitrocellulose rather than paraffin is used, less distortion occurs Contrary to Lumb,[37] there is no need to cut sections thinner than 6 μ. Eosin-azure or any other convenient Romanovsky stain should be employed after a light preliminary stain with hematoxylin. The addition of silver impregnation for reticular fibers may be an aid in demonstrating tissue architecture.[37]

Autopsy material, unless obtained within minutes after the patient's death, will not yield good cytologic detail even though architectural features are preserved. Concepts based upon the interpretation of fine morphologic detail in tissues obtained at autopsy should be viewed with caution

Many other techniques have been advocated to demonstrate adequately cytologic detail. Some, such as peroxidase and oxidase stains, have fallen into disrepute More recently smears made from aspirated tissues or from the cut surface of biopsy specimens have been utilized This approach has been predicated on the belief that cytologic detail has been better delineated in smears than in sections.[4, 5 38, 54] This contention is not valid provided that the sections are prepared by an adequate technique. On the other hand the smear method has had the following obvious disadvantages.[8 43] (1) it fails to demonstrate architecture, (2) the numerical interrelationships of the cells are disturbed in the process of making the smears, and (3) there is no means of determining whether the area of a pleomorphic tissue which has been smeared is representative In regard to the last objection, the smear technique has been particularly unreliable in evaluating the relative amounts of fat, myeloid, and lymphatic tissue in the marrow[8, 43] Based on my own somewhat limited experience, as well as on a review of the literature, I believe there has been no new information of value resulting from the smear technique

Some techniques are still too new to evaluate The study of enzyme systems[16] and phase and electron microscopy merit further consideration

Criteria of Response to Therapy

The major purpose of this investigation is to correlate histopathology with prognosis and response to therapy Accordingly, it is appropriate to define precisely what is meant by a good and poor response to therapy At the out-

set, it must be emphasized that as long as cells sensitive to radiation, chemotherapeutic agents, or steroids are present in malignant tissues, treatment with any of these agents will result in a shrinkage of palpable masses and a fall in the patient's white blood cell count without necessarily producing a favorable clinical response.[9-13] Consequently these indexes are not valid criteria of a favorable response to therapy.

A more logical and meaningful concept is that amelioration or regression of *signs and symptoms which interfere with the patient's normal way of life* may be considered as evidence for a favorable clinical remission. These signs and symptoms may be divided into systemic, hematopoietic, and local evidences of activity of the disease and may be utilized as an indication for treatment. Systemic symptoms are exemplified by chills, fever, malaise, anorexia, and weight loss. Hematopoietic abnormalities include lack of production of cells and platelets at a normal rate or a decrease in survival of these structures in the peripheral blood Local manifestations result when a mass exerts pressure upon or interferes with the function of a tissue or organ in which it is located, or exerts a similar effect upon adjacent structures.

It is evident that if the foregoing criteria are accepted, many patients have been subjected to treatment when they were in an asymptomatic state. What has been considered a remission, therefore, has often merely consisted of the shrinkage of a node or group of nodes or of a fall in white blood cell count occurring as a result of the particular treatment, without improvement in the patient's prognosis or condition

ANALYSIS OF PROGNOSIS AND RESPONSE TO THERAPY

Giant Follicular Lymphoma

Newer Concepts of Classification

Our concepts concerning this disease have altered considerably. At first, giant follicular lymphoma was considered to be a benign hyperplasia, later, it was thought to be a somewhat benign lymphoma Gall was the first to recognize that not all cases fulfilling the morphologic criteria for giant follicular lymphoma had the same prognosis or response to therapy [24] He recognized four types based upon the relative number of small lymphocytes, medium lymphocytes, and reticulum cells in the follicles and upon the degree of preservation of the follicular architecture His Type I, characterized by nodules composed of small lymphocytes, tended to develop toward Type IV, the follicles became less distinct and anastomosed with each other Both changes coincided with a progressive tendency toward a more unfavor-

able clinical course and with a poorer response to therapy. These concepts have been in general verified by Lumb,[37] Bilger,[7] Wright,[59] and Baggenstoss.[3]

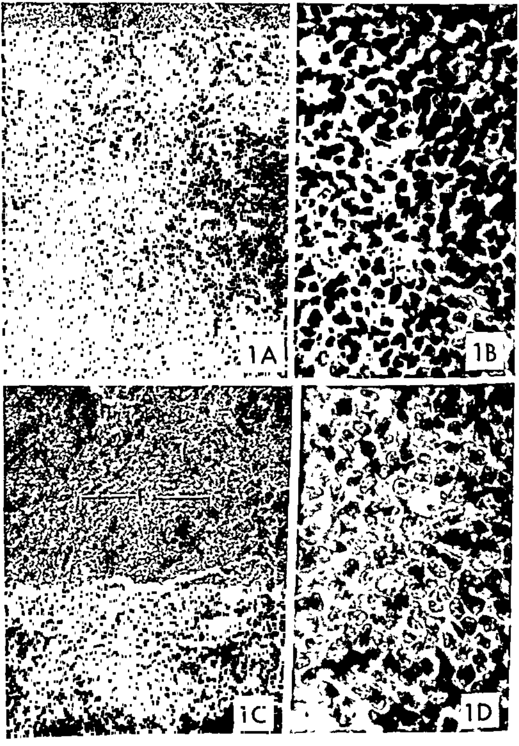
Rappaport and colleagues[45] in a more recent paper, advocated a slightly different interpretation. He recognized the four general types described by Gall and added a fifth, or Hodgkin's type In addition, Rappaport believed that the lymph nodes lost their follicular pattern and retained the characteristic dominant cell type. For example, a Type I node, composed of small lymphocytes, will lose its follicular pattern and evolve into lymphocytic lymphosarcoma. Similarly, a Type IV node composed of reticular cells, if it loses its follicular pattern, takes on the characteristics of a reticulum cell sarcoma. The latter has a poor response to therapy and thus a poor prognosis.

Cytologic Varieties

My own experience with giant follicular lymphoma has indicated that all these varieties have occurred and that, further, the prognosis and response to therapy have been related to the histopathology at the time of biopsy Figure 17-1*A* and *B*, from a node biopsy in 1913, was an example of the most benign type (Type I of Gall and Rappaport) in which the follicles were composed predominantly of dense sheets of small lymphocytes. The interfollicular tissue also consisted of small lymphocytes in slightly reduced concentration. The patient had been treated with nitrogen mustard (HN_2) in 1943 and 1944 in spite of a lack of activity of his disease. In 1948 he received x-ray therapy because of a swollen right leg.

The follicular pattern was more clearly demonstrable in a node obtained by biopsy in 1950 than it was in the specimen studied in 1943 (Fig. 17-1*C* *vs.* 17-1*A*). However, the lymphocytes were larger and more delicately stained than those in the node removed earlier (Fig. 17-1*B* *vs.* 17-1*D*) Examination of splenic tissue obtained by needle biopsy within a day of last node biopsy revealed numerous large follicles similar to those in the node. In both, the predominant cell more clearly resembled a medium than a small lymphocyte.

This patient pursued a gainful occupation until 1950 From that time on, he required increasingly frequent treatment until 1955. Thereafter he was unable to work until his death in December, 1956, with generalized lymphadenopathy and hepatosplenomegaly. In retrospect one wonders whether his favorable course from 1943 to 1950 resulted from the nitrogen mustard he received in 1943 According to Cocchi,[17] the longest remissions have followed surgical extirpation of nodes Nevertheless, in his paper he referred to patients who survived equally long and had never been treated. Accord-


1A
1B
1C
1D

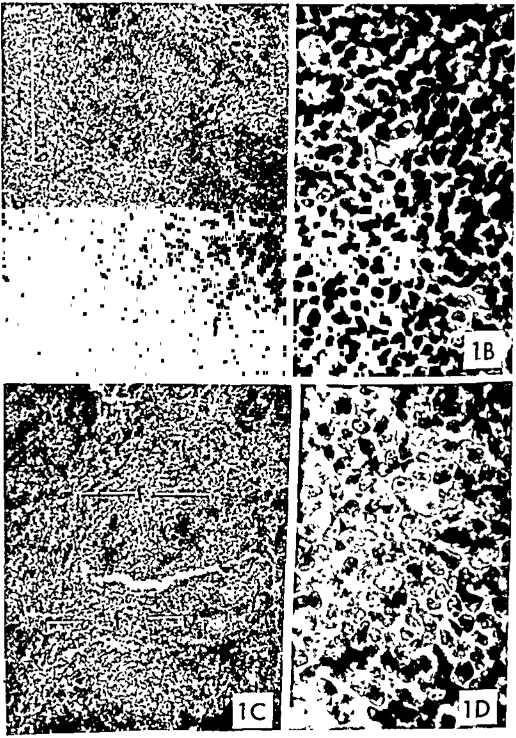
ing to Diamond,[21] the length of time the patient lived was not affected by specific therapy. Case 1 was an example of the most benign type of giant follicular lymphoma. The patient's clinical course illustrated that the best response to therapy occured when indications for therapy were at best minimal and that the response to therapy became poorer with a change in cytology toward a more immature cell type. Contrary to Bilger's[7] and Lumb's[37] concept, my data supported the view that a change to a more malignant, treatment-resistant type of disease did indeed occur without loss of follicular pattern.

Case 2 represents the other extreme in the variegated pattern that is characteristic of the group of giant follicular lymphoma. The follicular pattern was well delineated (Fig. 17-2*A*). There was no evidence of diffuse sarcomatous change or of loss of follicular pattern that has been alleged to indicate a more malignant course [7, 37, 59] The follicles were ringed by small lymphocytes, but most of the inner core was composed of a pleomorphic mixture of reticulum cells and large and medium-sized lymphocytes (Fig 17-2*B*) This patient did not respond to x-ray therapy or nitrogen mustard and died within a few months of onset of his disease On the basis of this case, it would appear, in accord with Rappaport and colleagues,[45] that cytologic composition was more important than the architectural pattern as a guide to prognosis and response to therapy

Case 3 had a fulminating course, characterized by failure of the disease to respond to chemotherapy or to radiophosphorus. The patient's major clinical problems were hepatosplenomegaly, intractable ascites, and pleural effusion. Figure 17-3*A* demonstrates in a liver biopsy the typical follicular pattern, similar to that noted in the biopsy of lymph node and spleen The dominant cell type (Fig. 17-3*B*) was larger and more delicate than in the first biopsy of Case 1 (Fig 17-1*B*) and was smaller, more heavily stained, and with less conspicuous nucleoli than the large lymphocytes in Case 2 (Fig 17-2*B*) This cell is classified as a medium lymphocyte (lymphoblast) In this patient it was difficult to determine whether his malignant course and lack of response to therapy depended upon the massive hepatic and splenic involvement (local manifestations of activity of his disease) or upon the cell type characteristic of his lesion. Certainly a follicular pattern in Cases 2 and 3 was not correlated with the relatively favorable prognosis usually associated with giant follicular lymphoma

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FIG 17-1 *Case 1*, giant follicular lymphoma (*A*) Lymph node, 1943, poorly demarcated follicles (*F*) separated by less lymphocyte rich interfollicular tissue (× 80) (*B*) Small lymphocytes representing dominant cell type in follicles of *A*. (× 650) (*C*) Lymph node, 1950, follicles (*F*) more clearly demonstrable 7 years after original biopsy (× 80) (*D*) Follicles of *C* composed primarily of medium sized lymphocytes (× 650)


1B
1C
1D

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FIG. 17 2 *Case 2*, giant follicular lymphoma (*A*) Lymph node, well-demarcated follicles (× 80) (*B*) Center of follicle of *A*, composed of large (*L*) and medium sized lymphocytes (*M*) and reticulum cells (*R*) (× 650)

CHANGES IN HISTOPATHOLOGY

Case 4, similar to Case 1, illustrated another point, namely, the need for repeated biopsies if the physician is to keep pace with changes in the biology of the patient's disease. The patient was asymptomatic when seen originally in May, 1957, for a routine check-up, at that time the spleen extended to the umbilicus. There was no lymphadenopathy and peripheral blood counts were normal. Figure 17-4*A*, from a marrow biopsy, shows a large nodule

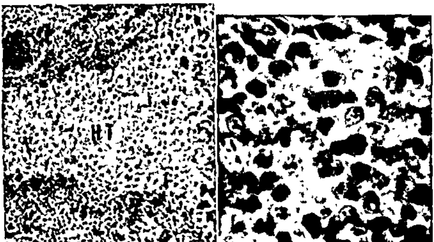


FIG 17 3 *Case 3*, giant follicular lymphoma (*A*) Liver, follicles separated by hepatic tissue (*HT*) (× 80) (*B*) Follicle of *A* composed primarily of medium-sized lymphocytes (× 650)

resembling that seen in the node of Case 2 (Fig. 17-2). The rest of the specimen consisted of hyperplastic myeloid tissue As in Case 2, there was a peripheral ring of small lymphocytes and a pleomorphic central core like a normal follicle in the cortex of a lymph node (Fig. 17-4*B*). The center of the follicle also contained nuclear debris, a rather unusual finding in giant follicular lymphoma but one which Wright does not consider a contraindication to this diagnosis

The patient was not treated In February, 1959, he developed fever,



FIG 17-4 *Case 4*, giant follicular lymphoma (*A*) Bone marrow, 1957, junction of large follicle and myeloid tissue (*MT*) (× 80) (*B*) Edge of follicle of *A*, composed of small (*S*), medium sized (*M*), and large lymphocytes (*L*) with prominent reticulum cells (*R*) and nuclear debris (*D*) (× 650)

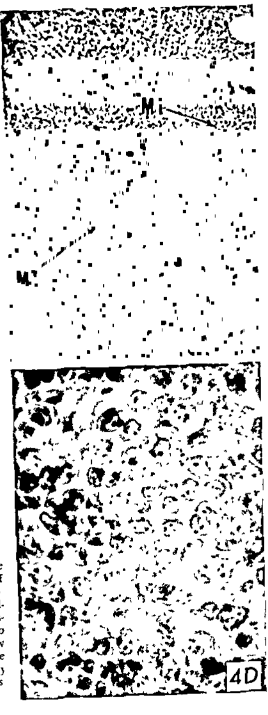


FIG 17-4 (*Contd*) (C) Bone marrow, 1959, large islands of anaplastic lymphatic tissue separated by smaller areas of moderately hyperplastic myeloid tissue (*MT*), artifact due to admixture of blood and marrow (*A*) (× 80) (*D*) Lymphatic tissue of Fig 17-3C, composed primarily of medium-sized lymphocytes (× 650)

malaise, anorexia, lymphadenopathy, anemia, and a white blood cell count of 30,000 with a differential count showing primarily medium-sized lymphocytes. A biopsy at this time (Fig. 17-4*C*, *D*) demonstrated that about three quarters of the marrow was composed of sheets of monomorphous medium-sized lymphocytes identical to those seen in the peripheral blood of acute lymphatic leukemia. The patient failed to respond to prednisone therapy and died in June, 1959

Evaluation of Histopathology

From a synthesis of my own experience and that in the literature, it would seem that when the dominant cell type was a small lymphocyte and the follicular pattern well preserved, prognosis has been most favorable and therapy was probably not indicated.

As the dominant cell type shifted to a medium-sized lymphocyte or reticulum cell, both prognosis and response to therapy became poorer. Involvement of the marrow and a consequent change to subacute or acute leukemia indicated a poor response to therapy and a poor prognosis Anday and Schmitz[1] have recorded the only case in which a transition to leukemia reversed spontaneously or following therapy. Since a change to a more malignant pattern may occur at any time, serial biopsies afford the most satisfactory means of assessing the patient's status

Chronic Lymphatic Leukemia

Classic Concept

According to classic description, chronic lymphatic leukemia is a disease characterized by hepatosplenomegaly, lymphadenopathy, anemia, thrombocytopenia, and marked leukocytosis due to an absolute rise in the lymphocyte count In this type of case, Minot and Isaacs[40] reported an average survival of three to five years both in patients receiving radiation therapy as well as in those not given specific therapy Much more recently, Tivey[57] has reported a somewhat shorter average survival, adding that longevity in chronic myelogenous leukemia and chronic lymphatic leukemia is comparable This comparatively poor prognosis in chronic lymphatic leukemia noted by Tivey has not been consistent with my observations By including all patients with chronic lymphatic leukemia in a single category, this author has not made due allowance for the prognosis for the individual patient as determined by the biology of *his* disease It is little wonder that Osgood,[20, 42] whose patients formed the basis for Tivey's study, recommended the same type of therapy for all patients regardless of the type of chronic lymphatic leukemia manifested

Classification of Response and Prognosis in Terms of Severity of Disease

We are now in a position to recognize an early, asymptomatic stage in chronic lymphatic leukemia[20, 44, 57] primarily as a result of the fact that blood counts are now obtained on all hospitalized patients and biopsy of the bone marrow is usually performed whenever an absolute lymphocytosis is noted. The diagnosis of chronic lymphatic leukemia under such circumstances was usually fortuitous Pisciotta and Hirschboek,[44] for example, noted that 38 per cent of all the patients with chronic lymphatic leukemia in his series were diagnosed while under investigation for another problem.

Patients with early benign chronic lymphatic leukemia have minimal lymphadenopathy or hepatosplenomegaly. There is no anemia, only slight thrombopenia, and moderate leukocytosis. They form a group in which any type of cytocidal treatment will give an excellent response as evidenced by a fall in white blood cell count and shrinkage of masses; in retrospect one may ask whether treatment was indicated or was of any real benefit [20]

The histopathologic hallmark of the benign type of chronic lymphatic leukemia was a marrow which contained occasional islands of lymphatic tissue, composed of normal, small, heavily stained lymphocytes with a poorly demonstrable reticular cell background. Mitoses were not seen, there was no follicular pattern manifested by the lymphatic tissue, and a sharp border between the lymphatic and myeloid tissue was recognizable. The latter was normal in amount and distribution of cells and lineages and had a normal amount of tissue iron.

This benign type of chronic lymphatic leukemia is illustrated by Case 5 The patient was a 70-year-old nurse who was known to have had normal white blood cell counts in 1949 and 1951, in each instance the count was obtained following an injury. In 1953, at a routine examination, a white blood cell count of 14,000 with 80 per cent small lymphocytes and a normal hemoglobin was discovered Marrow biopsy revealed a normal amount of myeloid tissue (Fig 17-5*A*) with an occasional small island of dense lymphatic tissue, each of which was composed of a sheet of small lymphocytes —hardly more than is seen normally in many elderly patients [14]

By March, 1959, her white blood cell count without treatment had risen to between 25,000 and 35,000 with 80 per cent small lymphocytes She remained asymptomatic with normal hemoglobin and platelets The only abnormality on physical examination was a 1 cm node in her left axilla. Figure 17-5*B*, taken from a section of bone marrow obtained in March, 1959, demonstrates a large island of lymphatic tissue and normal myeloid tissue The lymphatic tissue was composed predominantly of dense sheets

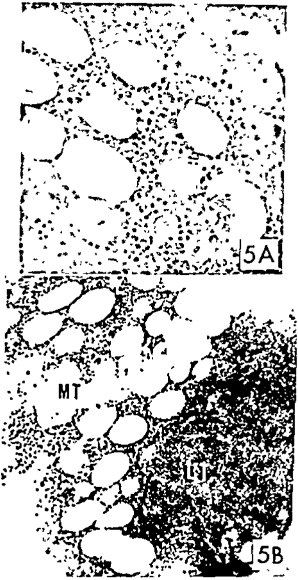


FIG 17-5 *Case* 5, chronic lymphatic leukemia (*A*) Bone marrow, 1953, area of normal myeloid tissue (× 100) (*B*) Bone marrow, 1959, large island of lymphatic tissue (*LT*) sharply demarcated from normal myeloid tissue (*MT*) (× 80)

of small lymphocytes There was lymphatic metaplasia of the area between the nearest fat spaces (Fig 17-5*D*) adjoining the lymphatic nodule However, there still was adequate myeloid tissue (Fig 17-5*C*). Had this patient been treated in 1953, the 6-year "remission," which would have been assumed, would have been considered a therapeutic triumph

Case 6 represents a common situation, that of a patient with fairly advanced chronic lymphatic leukemia At the age of 73 in 1950, the patient noted lymphadenopathy and mild purpura In 1952 physical examination



FIG. 17.5 (*Contd.*) (*C*) Residual normal myeloid tissue of *B* (× 650) (*D*) Lymphocytic metaplasia of tissue between fat cells separating lymphatic nodule from myeloid tissue of *B*. (× 650)

revealed mild (1 cm.) generalized lymphadenopathy but no splenomegaly. The red blood cell count was 4.3 million, reticulocytes were 2 per cent, and at least 90 per cent of the 101,000 white cells were small lymphocytes. In June, 1953, she inadvertently received one dose of 3 mc. of radiophosphorus, lowering her white blood cell count from 112,000 to 60,000 without any other change in her clinical status. She continued asymptomatic until June, 1956, when she had an episode of cellulitis involving her left foot. Her hemoglobin was 13.7 and the white blood cell count 211,000. From August, 1956, to date, she has received Gantrisin for chronic cystitis. In May, 1958, she had carious teeth removed without incident. She developed an asymptomatic anemia with a hematocrit of 31 per cent for the first time in October, 1958, when she was admitted with phlebitis. Her white blood cell count was 237,000, and there was a slight thrombopenia. The spleen was enlarged 4 cm below the costal margin and the liver 2 cm. below the costal margin.

Figure 17-6 is from a marrow biopsy obtained in October, 1958, showing the dense, diffuse, small lymphocytic metaplasia characteristic of most of the tissue in the biopsy. There were isolated myeloid cells (Fig 17-6*C*), obviously enough to produce almost a normal number of red cells, granulocytes, and platelets.

Case 7 is an example of a more fulminating course. The patient was 58 years of age in January, 1957, when he noted weight loss and generalized lymphadenopathy From February, 1957, to August, 1958, he was treated intermittently with local x-ray therapy, transfusions, and chlorambucil, but at no time did he ever improve enough to resume a gainful occupation, even though his total white blood cell count was kept near normal

When first seen in August, 1958, the patient was pale, emaciated, febrile, and dyspneic He had generalized lymphadenopathy, slight hepatosplenomegaly, a hemoglobin of 6 2 gm , and a white blood cell count of 6,600 with 100 per cent small lymphocytes He was treated only by transfusions until December, 1958, when his white blood cell count rose to 115,000 His condition was unaltered in other respects

Figure 17-7 is from a surgical biopsy of the sternum resorted to after an adequate specimen was not obtained by three needle biopsies As in Case 6, (Fig 17-6*A*), there was an apparent replacement of the myeloid marrow by dense sheets of small lymphocytes However, unlike Case 6, higher power magnification (Figs 17-6*B* and *C vs* 17-7*B*) failed to reveal any myeloid cells, suggesting a much poorer prognosis Severe hemosiderosis, in part due to prior transfusions, was noted The patient failed to respond to prednisone, nitrogen mustard, and local x-ray therapy and died in April, 1959. During the last few months of his life, he had a positive direct Coombs test and

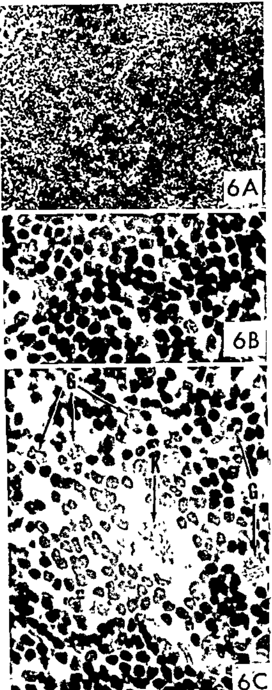


FIG 17 6 *Case 6,* chronic lymphatic leukemia (*A*) Bone marrow, with apparent complete replacement of myeloid by lymphatic tissue (× 80) (*B*) Same biopsy as *A*, dense lymphatic tissue without myeloid cells (× 650) (*C*) Another area in same biopsy as *A*, megakaryocytes (*K*) and isolated myelocytes (*G*), between small lymphocytes (× 650)

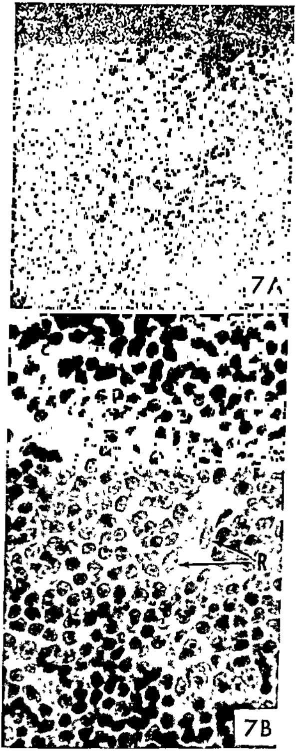


FIG 17-7 *Case 7*, chronic lymphatic leukemia (*A*) Myeloid tissue completely replaced by dense lymphatic tissue (× 80) (*B*) Small lymphocytes with occasional reticulum cells (*R*) but no myeloid tissue (Higher power of *A*, × 650)

needed two transfusions a week to maintain his hemoglobin between 6 and 10 gm.

Hyperhemolytic Syndrome

Since the introduction of suitable techniques for determination of red cell survival, a group of severely anemic patients with hyperhemolysis, representing 10 per cent of all cases of chronic lymphatic leukemia, has been recognized[22, 44, 57] Frequently the anemia is aggravated and the red cell survival shortened by cytocidal therapy.[22] These patients may respond to steroids; my own results have been unpredictable In general, patients with a positive Coombs test have responded better than those with a negative Coombs test.

Figure 17-8 is taken from Case 8, a patient with chronic lymphatic leukemia with an extremely severe anemia and a very large spleen; both of the latter were out of proportion to what would usually be expected in a patient with chronic lymphatic leukemia whose white blood cell count was only 30,000. The anemia was demonstrated to be hemolytic in type. Transfused genetically compatible red cells were completely destroyed in two hours.

The patient's brother also had chronic lymphatic leukemia with a white blood cell count of 30,000 and a hemoglobin of 16 gm He was asymptomatic and did not have lymphadenopathy or hepatosplenomegaly His marrow resembled that of Case 5 (Fig 17-5*B*, *C*, and *D*)

The patient's marrow (Fig 17-8*A* and *B*) had only a few islands of lymphatic tissue and was similar in this respect to her brother's as well as to that seen in Case 5 Such a finding would ordinarily signify a relatively benign disease. However, the remaining myeloid marrow had marked panhyperplasia of the three major marrow lineages and of plasma cells (Fig. 17-8*A* and *C*) Upon further investigation a macroglobulin was demonstrated The patient did not develop a remission following x-ray therapy to the spleen, although her spleen (Fig. 17-8*D*), which had a fairly normal architecture, was reduced in size and her white blood cell count fell to normal After treatment with prednisone, the concentration of the macroglobulin decreased, the spleen became still smaller, the white blood cell count remained normal, and the patient was able to resume her duties as a housewife. The survival time of transfused compatible cells rose to fourteen days

Evaluation of the Role Played by the Marrow

These cases illustrated the need to evaluate two interrelated factors in chronic lymphatic leukemia, namely, the amounts of lymphatic and of myeloid tissue The mildest cases, i e., those not needing treatment, were characterized by a fairly normal amount of myeloid marrow There were

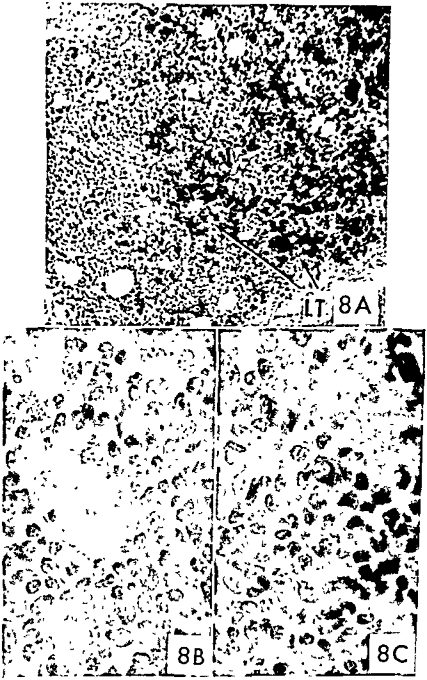


FIG. 17.8 *Case 8*, chronic lymphatic leukemia (*A*) Bone marrow, markedly hyperplastic myeloid tissue with occasional areas of lymphatic tissue (*LT*) (× 80) (*B*) Island of lymphatic tissue composed of small lymphocytes from *A* (× 650) (C) Myeloid tissue from *A* (× 650)

occasional small islands of lymphatic tissue composed of dense sheets of small lymphocytes without any follicular pattern Mitoses were usually not demonstrable. The lymphatic tissue infiltrated only between the neighboring row or two of fat cells and not into the myeloid tissue. Over the course of years, these islands of lymphatic tissue have slowly increased in number. There was adequate space for expansion of the lymphatic tissue into fat



FIG 17 8 (*Contd*) (*D*) Spleen, normal architecture with clearly demonstrable white pulp (*WP*) and cords and sinuses in the red pulp (*RP*) (× 80)

without infringing on normal myeloid tissue which usually occupies only 20 to 30 per cent of the sternal marrow volume in the age group in which chronic lymphatic leukemia occurs

There were patients with disease of intermediate severity between the mildest form (Case 5) and the most advanced and progressive type (Case 7) The transition from a less to a more severe form was marked by a gradual increase in lymphatic tissue which eventually, after replacing most of the fat, replaced the myeloid tissue

Although every patient whose marrow had had a histopathology similar to

that in Case 5 (Fig. 17-5) has had a benign course, the reverse was not necessarily true. There was really very little difference between the marrows of Cases 6 (Fig. 17-6) and 7 (Fig. 17-7), even though in the former the marrow was able to produce a fairly normal number of peripheral blood cells In contrast, the marrow in Case 7 was severely deficient, at least in erythropoietic capacity. Since there was only a moderate decrease in red cell survival, excess hemolysis was not the major factor in the severe anemia in this patient. Patients with apparently marked replacement of myeloid tissue may still have functionally adequate marrows (Fig. 17-6). Further, an extreme degree of replacement of myeloid by lymphatic tissue has been found at autopsy in patients who have had chronic lymphatic leukemia and have died of an unrelated cause at a time when their hemoglobin level was fairly normal.[46]

An example of the pitfalls encountered when one attempts to predict the ability of a patient to form red cells on the basis of histopathologic examination of the marrow was illustrated by the case of an elderly woman presenting with symptoms of severe plethora.[6] She had a hemoglobin level of 28 gm and a corresponding elevation of red cell volume. Biopsies of her marrow, liver, and spleen failed to demonstrate that these tissues differed from those of any patient with moderately advanced chronic lymphatic leukemia, such as Case 6, except for absence of tissue iron The latter, of course, represented a diversion of iron from tissue stores into the expanded hemoglobin mass incident to the polycythemia [62]

Evaluation of Histopathology of Tissues Other Than Marrow

Only sporadic attempts have been made to take biopsies of other organs [9, 10, 13] Lymph node tissue has not provided a useful index of prognosis or therapeutic response. Liver biopsies have not been a reliable index to therapeutic response either because of the limitations in size of the biopsy or because the liver is an unsatisfactory area for study Similarly, spleen biopsies have not proved especially useful. If the spleen, liver, or nodes were filled with small lymphocytes and were not too fibrous, any type of cytocidal therapy would reduce their size and concomitantly lower the white blood cell count without necessarily inducing a remission in the sense defined in this chapter [13] In general, but not universally, in the cases in which treatment had been effective, there has been a lesser degree of lymphocytic proliferation and of architectural distortion in the liver and spleen (Fig. 17-8*D*) than in those in which treatment had been ineffective

Effect of Treatment on the Natural History

It is apparent from a review of the response to therapy of patients with chronic lymphatic leukemia that patients who have experienced the most

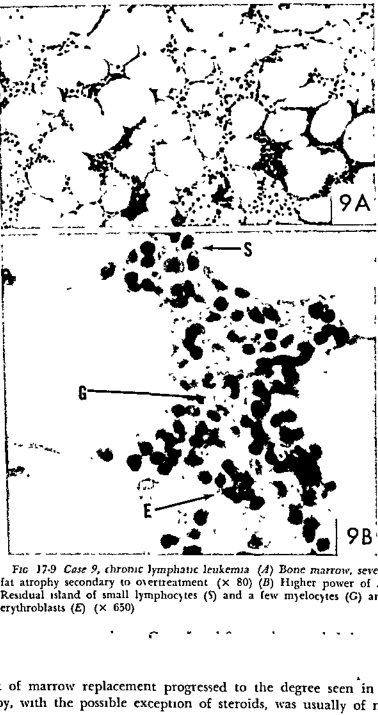


Fig 17-9 *Case 9*, chronic lymphatic leukemia (*A*) Bone marrow, severe fat atrophy secondary to overtreatment (× 80) (*B*) Higher power of *A* Residual island of small lymphocytes (S) and a few myelocytes (G) and erythroblasts (*E*) (× 650)

extent of marrow replacement progressed to the degree seen in Case 7, therapy, with the possible exception of steroids, was usually of no avail,

that in Case 5 (Fig. 17-5) has had a benign course, the reverse was not necessarily true. There was really very little difference between the marrows of Cases 6 (Fig. 17-6) and 7 (Fig. 17-7), even though in the former the marrow was able to produce a fairly normal number of peripheral blood cells In contrast, the marrow in Case 7 was severely deficient, at least in erythropoietic capacity. Since there was only a moderate decrease in red cell survival, excess hemolysis was not the major factor in the severe anemia in this patient. Patients with apparently marked replacement of myeloid tissue may still have functionally adequate marrows (Fig. 17-6) Further, an extreme degree of replacement of myeloid by lymphatic tissue has been found at autopsy in patients who have had chronic lymphatic leukemia and have died of an unrelated cause at a time when their hemoglobin level was fairly normal.[46]

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It is apparent from a review of the response to therapy of patients with chronic lymphatic leukemia that patients who have experienced the most

formly refractory to treatment, the patient dying of pancytopenia and not leukemia

Acute Lymphatic Leukemia

By the time a patient with this disease is first seen, the marrow has already been replaced by anaplastic lymphatic tissue (Fig. 17-10), and there was usually a minor degree of palpable enlargement of lymph nodes, liver, and spleen. In some instances the dominant cell is so immature as to make impossible differentiation from acute stem cell or myelogenous leukemia. At this point diagnosis rests upon the somewhat illogical approach of "the company they keep"

Case 10 was an 18-year-old man who presented with fever, malaise, weakness, and a hemorrhagic diathesis. He failed to respond to steroid therapy, Aminopterin, or 6-mercaptopurine and lived about 6 months following diagnosis The normal myeloid tissue was almost completely replaced by a monomorphous sheet of medium-sized lymphocytes (Fig 17-10) These cells were so numerous as to obscure all other cell types

Although it was impossible to determine on the basis of the histopathology whether the patient was an adult or a child, the remission rate after steroids is much higher in children than in adults, nor may one predict from the histopathologic findings which patient will obtain a remission from folic acid antagonists, steroids, or 6-mercaptopurine

Lymphosarcoma

Definition

Under this heading (Figs 17-11, 17-12) were included all lymphomas in which the predominant cell type was a lymphocyte and in which the number of lymphocytes was sufficient to obscure the architecture of the involved organ. When there was invasion through the capsule or metaplasia of surrounding extracapsular tissue, the extracapsular malignant tissue had the same cytology and architecture as that within the lymph node. Marrow involvement was absent or minimal, and there was no invasion of the peripheral blood in early stages of the disease

Cytologic Pattern

Case 11 was a 52-year old electrician who first noted generalized lymphadenopathy in 1951 In August, 1953, while asymptomatic, he was given nitrogen mustard at another institution. When first seen by me in January, 1954, he had generalized lymphadenopathy, and the spleen and liver were both enlarged 3 cm below the costal margin The peripheral blood was

although the white blood cell count has invariably been made to fall. As long as small lymphocytes were the predominant cell, any form of cytocidal therapy given in adequate dose has successfully reduced the size of abnormal masses and lowered the white cell count by destroying lymphatic tissue but has not necessarily produced a remission in the sense herein defined.[11]

Occasional cases under continuous therapy have had very prolonged and benign courses.[46] Similar results have been noted in patients without treatment.[39] Often the first evidence of anemia in a previously benign type of chronic lymphatic leukemia has followed radiation or chemotherapy.[31] It is obvious that we need more knowledge of the natural history of untreated lymphatic leukemia. It is equally obvious that one cannot evaluate the effects of treatment on patients with chronic lymphatic leukemia without first de-

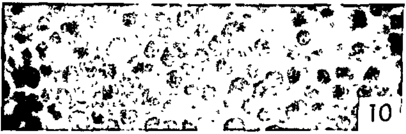


FIG. 17-10 *Case 10*, acute lymphatic leukemia Bone marrow composed primarily of monomorphous medium-sized lymphocytes (× 650)

termining the extent of marrow involvement in each patient Considering all patients with chronic lymphatic leukemia as a single group will efface the very real differences between individual patients

OVERTREATMENT

The subject of the response to therapy cannot be left without consideration of *the effects of overtreatment* [11, 47] *Case 9*, a 60-year-old woman, had lymphatic leukemia which was discovered accidentally in 1957 when she consulted her physician because of anemia due to bleeding hemorrhoids She was overtreated with x-ray therapy and radiophosphorus in an attempt to burn out the disease, pancytopenia, refractory to all treatment, resulted The patient became a hopeless invalid and required from 5 to 10 transfusions a month for support Figure 17-9*A* is a biopsy of the patient's marrow to show the serous fat atrophy and severe hemosiderosis which resulted from overtreatment There was an almost complete absence of myeloid and lymphatic tissue A few islands of erythroblasts and small lymphocytes were demonstrable Serous fat atrophy, such as that seen here, has often followed overzealous treatment It signaled an extremely poor prognosis and was uni-

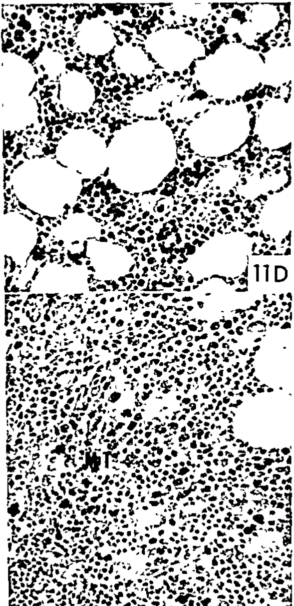


FIG 17-11 (*Contd*) (*D*) Bone marrow, normal myeloid tissue, no evidence of lymphatic metaplasia, January, 1954. (× 160) (*E*) Bone marrow, lymphatic metaplasia (*LT*) and hyperplasia of myeloid tissue due to hypersplenism, July, 1957 (× 160)

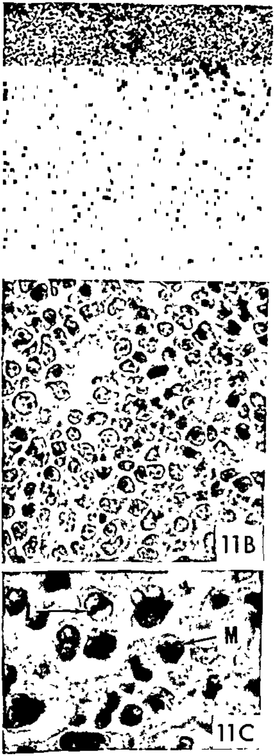


FIG 17 11, *Case II*, lymphosarcoma (*A*) Lymph node, typical replacement of normal architecture by sheets of small lymphocytes, January, 1954 (× 80) (*B*) Higher power of *A* Small lymphocytes, dominant cell type (× 650) (*C*) Lymph node, obtained at autopsy, dominant cell types are medium-sized (*M*) and large lymphocyte (*L*), January, 1958 (× 650)

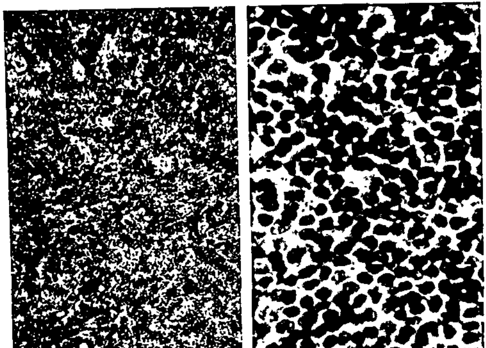


FIG 17-12 *Case 12*, lymphosarcoma (*A*) Lymph node, replacement of architecture by dense lymphatic tissue similar to Fig 17-11*A* (× 80) (*B*) Higher power of Fig 17-11*A*. Small lymphocyte is dominant cell type as in Fig 17-10*B*. (× 650) (*C*) Bone marrow, marked replacement of myeloid tissue by small lymphocytes (× 650)

normal. Figure 17-11 is from a biopsy of a lymph node taken in January, 1951. It illustrates the typical diffuse lymphatic tissue in which all architectural pattern was obscured by small lymphocytes.

Because of subjective complaints of weakness and symptoms related to pressure of nodes, he was treated with P^{32} intermittently through 1951 and 1955 but received no specific therapy thereafter until November, 1956; during this period he worked as a uranium prospector. He did not miss any more time from work than the average man of similar age. The patient's clinical course was therefore typical of lymphocytic lymphosarcoma in that most of these patients live from four to six years [45]

Occasionally a patient with lymphosarcoma characterized by small lymphocytes will exhibit an unusually benign course. I am now following a 50-year-old woman whose diagnosis was made by femoral node biopsy in 1953 and verified by another lymph node biopsy in 1955 This patient has never been treated and as of August, 1959, was carrying on an active life and working as a waitress In March, 1959, she survived a radical mastectomy with postoperative pneumonia and atelectasis requiring emergency tracheostomy. In another woman of similar age, diagnosis was first made in 1947 by node biopsy at which time she received one course of x-ray therapy. She had a splenectomy in 1953 for hemolytic anemia and also had a nephrectomy for a hypernephroma, discovered by chance at the same time. Without any further therapy she lived a normal life until April, 1959, when the hemolytic anemia recurred In neither of these patients did the findings in the lymph node biopsies explain why their disease has been so much more benign than the average case of lymphocytic lymphosarcoma

Much more rarely the dominant cell type in a patient with lymphosarcoma was a medium sized or large lymphocyte Some of the latter cases have been difficult to distinguish from reticulum cell sarcomas The response to therapy and prognosis has been increasingly poor with increasing size and delicacy of staining of the lymphocytes in biopsy specimens For example, a remission was rarely obtained and the patient usually died within a year when the lymphocytes were similar to those in Figures 17 3*B* and 17-1*C*

Other Factors

Although usually the type of lymphocyte that constitutes the dominant cell was the most important factor in determining response to therapy and prognosis, other factors occasionally became more important. Some of these were the following (1) specific sites of involvement by the lymphosarcoma, (2) hypersplenic syndromes, (3) the changing natural history which was usually a result of a change in cytology, and (4) various intercurrent affections such as coronary artery disease

Hypersplenic Syndrome. The best prognosis was found when the myeloid tissue in the marrow was most nearly normal, neither replaced by lymphoma or fat, nor hyperplastic because of a hypersplenic syndrome. When hyperplasia of myeloid tissue was accompanied by other evidence of autoimmune hemolytic disease (Fig. 17-11*D vs.* 17-11*E*), the response of the hemolytic component at least to prednisone or splenectomy was gratifying Radiation (either isotopic or x-ray) and chemotherapy were less effective in this variation of lymphosarcoma.

Case 11, characterized by a fairly benign course from 1950 to 1957, thereupon developed severe hemolytic anemia with a reticulocytosis, and an elevated indirect serum bilirubin. His marrow showed an increase in lymphatic tissue and, in addition, a severe erythroblastic hyperplasia The hemolytic syndrome responded very well to treatment with prednisone until July, 1957, when the patient again developed a reticulocytosis, indirect bilirubinemia, and anemia The marrow showed marked lymphatic metaplasia with hyperplasia of residual myeloid tissue (Fig 17-11*E*). Splenectomy was performed on October 4, 1957, with alleviation of the hypersplenic syndrome as manifested by a marked decline in the number of transfusions needed until the patient's death in January, 1958.

Change in Cytology. The hemolytic syndrome of Case 11 had responded first to prednisone and thereafter to splenectomy. However, following splenectomy, the biologic characteristics of his disease changed as manifested by the onset of fever, anorexia, increasing hepatomegaly and lymphadenopathy, and lack of response to therapy The nodes obtained at autopsy in January, 1958, were characterized by the predominance of large and medium-sized lymphocytes in marked contrast to the dense sheets of small lymphocytes that had been the dominant cell type when he had responded to irradiation earlier in the natural history of his disease (Fig 17-11*B vs.* 17-11*C*)

Intercurrent Disease. Case 11 had rather severe coronary arteriosclerosis. He experienced incapacitating angina at a level of 9 or 10 gm of hemoglobin, a degree of anemia to which most chronically ill patients are not sensitive Consequently therapy, first prednisone and later splenectomy, was necessary in contrast to other patients with a similar disease and severity of anemia

Monocytic Leukemia

This very vexing problem is an example of our subjective approach to cytologic diagnosis The incidence of this disease approximates 30 per cent of all acute leukemias according to those who use the supravital staining technique primarily; in contrast, other workers, such as Willis, are not convinced the disease exists or, at best, believe it to be a variant of acute lym-

Areas of Involvement by Lymphosarcoma. There were several regions in which, for unknown reasons, lymphosarcoma tended to remain localized. These were the skin, the orbit, and the stomach.[23, 30, 31, 37] Patients with lesions in these areas have done equally well with surgery or local x-ray therapy. One cannot but wonder if they would not have done as well if untreated, aside from the local effects of the lesions.

The rectal lymphoma may represent a biologically different, more benign entity even though it may show microscopic features of lymphosarcoma or giant follicular lymphoma.[27, 59] However, no patient with this lesion has been followed long enough to exclude possible spread to, or metaplasia of, other sites.

On the other hand other areas of involvement were correlated with a poor prognosis, primarily because of the nature of the site of the lesion, rather than because of the type of lymphosarcoma. Examples were intracranial or spinal cord lymphosarcoma with secondary neurologic disease and extension of mediastinal tumor into the lungs. In the latter, pulmonary drainage was impaired, and the patient fell prey to intractable pneumonitis.

The bone marrow was the most important of all extranodal sites of involvement As in other lymphomas, we are concerned with two interdependent factors, the amount of lymphatic and of myeloid tissue.

Lymphatic metaplasia has occurred at any stage in lymphosarcoma and has had an ominous prognosis in direct correlation with the degree of marrow replacement The node biopsy of Case 12 was morphologically similar to that of Case 11 (Figs. 17-11*A* and *B* *vs.* 17-12*A* and *B*) However, Case 12 also had severe pancytopenia, and surgical biopsy of his marrow (Fig 17-12*C*) demonstrated almost complete replacement of myeloid tissue by small lymphocytes, morphologically identical to those in his lymph node and in the node of Case 11 Case 12 failed to respond to therapy and is now being maintained on transfusions

More frequently a less fulminating course has occurred Over years, independently of therapy, the marrow underwent progressive lymphocytic metaplasia (Fig 17-11*D* *vs* 17-11*E*) The lymphocytes may retain their original cytology or may transform into medium-sized or large lymphocytes In the latter circumstance the prognosis and results of therapy were worse. According to Lumb,[37] this type of lymphatic metaplasia has occurred in about 15 per cent of all patients My own experience suggested not only a higher incidence but also indicated that in any patient the longer the patient survived, the more likely was metaplasia to occur. Frequently lymphatic metaplasia was accompanied by a leukemic blood picture in which the characteristic cell was a medium-sized lymphocyte [5] The prognosis and response to therapy were inversely related to the degree of marrow replacement and directly to the degree of maturation to the small lymphocyte



FIG 17-15 *Case 13*. Hodgkin's paragranuloma (*A*) Lymph node, fairly normal follicle (× 80) (*B*) Small lymphocytes (*S*) at edge of follicle, pleomorphic inner portion made up of medium sized and large lymphocytes, reticulum cells, and nuclear debris, rows of plasma cells (*P*) in medullary cords, medullary sinuses (*V*) are demonstrable (Higher power of Fig 17-14*A*, × 160)

phatic leukemia.[58] One of two events has transpired in every instance in which I have made the diagnosis of acute monocytic leukemia on the basis of smears of the peripheral blood or bone marrow. Either examination of the sections prepared by the Maximow technique demonstrated specific neutrophilic granules in the cells, or in the course of time, specific neutrophilic granules appeared in the cells even *in dry smears.* My own personal bias in this still bitterly debated subject is that there is no such entity as monocytic leukemia.

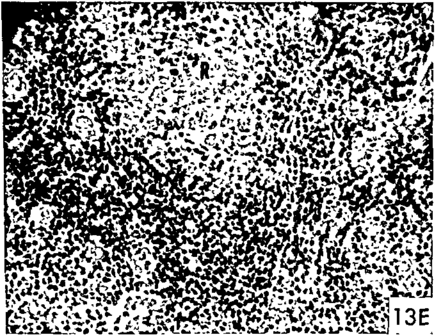
Hodgkin's Disease

Evaluation of Prognosis and Response to Therapy Based on Lymph Node Biopsy

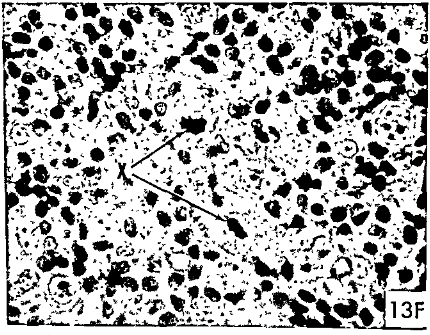
Since the classic monograph by Jackson and Parker[31] published in 1937, there has been almost universal acceptance of the fact that the lymph nodes are the site of three types of Hodgkin's disease, the paragranuloma, granuloma, and sarcoma.[19, 25, 26, 32, 33, 35-38, 45, 49, 60, 61] Investigators agreed that the paragranuloma had the best prognosis, the sarcoma had the poorest, and the granuloma, which is commonest, occupied an intermediate position. In my experience prognosis and prediction of response to therapy based upon the division into these three types on the first node biopsy will be accurate in about 80 per cent of cases

Paragranuloma. The paragranuloma is characterized by a dense, small lymphocytic infiltrate, by a variable degree of architectural effacement, and by the presence of Sternberg-Reed cells Rappaport and colleagues described in addition the presence of a follicular pattern early in the disease[45] According to Harrison, the lymph node may be divided into lobules by fibrous tissue, although most other authors believe that sclerosis is not a feature of the paragranuloma[26] Pleomorphism, irregular fibrosis, eosinophilia, neutrophilia, and foci of necrosis, all common in Hodgkin's granuloma, are not found[31]

Fig 17-13, from Case 13, is an untreated cervical node which appeared a few weeks prior to biopsy in a patient who had had Hodgkin's disease for 20 years, *the original biopsy showing a paragranuloma Many of the features* characteristic of the paragranuloma were still demonstrable despite the long duration of the disease There was preservation of normal architecture even to persistence of fairly normal appearing follicles and recognizable medullary cords and sinuses (Fig 17-13*A*, *B*) There were still areas very rich in lymphocytes (Fig 17-13*E*), and Sternberg-Reed cells were demonstrable (Fig. 17 13*F*) Unlike the paragranuloma, areas of prominent reticulum cells were *found,* similar to what Loew and Lennert[30] described, as the patient's



13E



X
13F

disease changed from a paragranuloma to a granuloma (Fig. 17-13*F*). The fibrosis may be construed as evidence of progression toward a granuloma. The increased number of plasma cells between the islands of lymphatic tissue (Fig. 17-13*B*) have been found in both paragranulomas and granulomas of long duration. In comparison to the original node, the node obtained in 1959 had a more irregular architecture, not as dense a lymphocytic infiltrate, and more scarring and plasma cells. This patient has since had a good re-

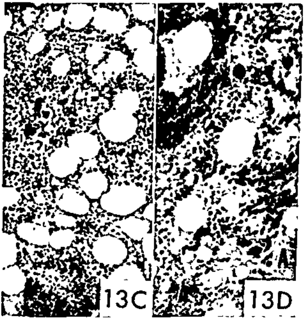
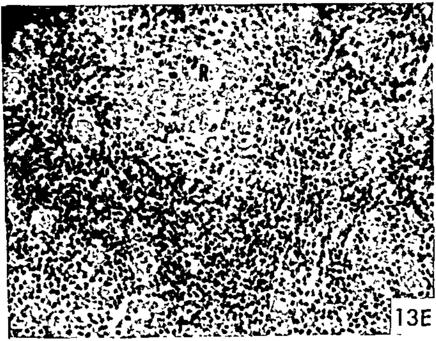


FIG. 17-13 (*Contd*) (*C*) Bone marrow, 1953, hyperplastic myeloid tissue (× 80) (*D*) Bone marrow, 1959, serous fat atrophy, artifact due to mixture of blood and marrow (*A*). (× 80)

sponse to x-ray therapy directed to an abdominal mass which caused gastrointestinal symptoms, chills, fever, and weight loss In the last 6 years, the duration of each treatment-induced remission has slowly decreased; this observation seems to be correlated with the change in the histopathology of the lymph nodes and marrow which will be described in more detail (see below).

The cytologic features of a paragranuloma indicate, as in chronic lymphatic leukemia and giant follicular lymphoma, that a good therapeutic response is to be expected and that the prognosis may be just as good if no treatment is given The one common denominator in all three diseases has



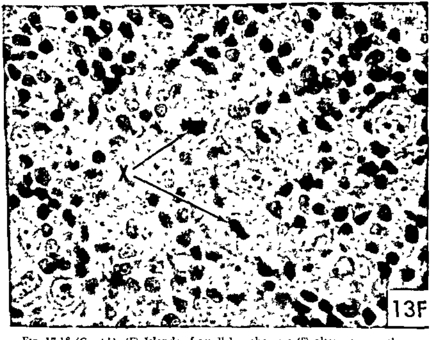


FIG 17-13 (*Contd*) (*E*) Islands of small lymphocytes (*S*) alternating with areas poor in lymphocytes with prominent reticulum cells (*R*) Same node as *A* (× 80) (*F*) Area poor in lymphocytes, with prominent reticulum cells and Reed Sternberg cells, 2 of which are in mitosis (*X*) Same node as *A* (× 650)

been the presence of many small lymphocytes, one of the most radiation-sensitive cells in the hematopoietic tissues [15]

The consensus is that a Hodgkin's paragranuloma indicates a survival of 8 to 20 years in the great majority of cases. During most of this time, the patients are essentially asymptomatic and are able to live a normal and even active physical life. Most of the patients reported have been treated solely on the basis of the diagnosis per se or of the presence of a palpable node; activity as herein defined has not been the indication for therapy. Occasionally, for various reasons some patients have remained untreated Wright described two such cases.[60] In one patient who was asymptomatic, recurrence of a node occurred 8 years after biopsy. The second patient was treated initially 7 years after the diagnosis was made, purely because of palpable nodes; at the time he was asymptomatic. Harrison's two untreated cases were still asymptomatic at 8 and 12 years after biopsy.[29] These four cases represent as good results as are reported in treated patients The possible deleterious effect of cytocidal therapy has recently been emphasized in the German literature in which the transformation of a benign Hodgkin's granuloma to a treatment-resistant Hodgkin's sarcoma has been ascribed to therapy.[32, 61]

Granuloma. Hodgkin's granuloma, representing about 80 per cent of all patients with Hodgkin's disease, occupies an intermediate position between sarcoma and paragranuloma As Jellife has pointed out, the morphologic pleomorphism parallels the marked variability in prognosis.[33] Some patients with a granuloma have had the benign course characteristic of a paragranuloma [36] Patients with this lesion have survived from less than 2 to more than 15 years, overlapping the paragranulomatous and sarcomatous varieties of Hodgkin's disease The great majority of patients have lived from 4 to 7 years after the diagnosis has been made

Figure 17-14 is taken from a previously untreated patient (Case 14) and shows a case intermediate between that of a granuloma and paragranuloma, *corresponding to Loew's group IIC* [35] *There were an unusually large* number of lymphocytes, but the eosinophilia, scarring, plasmacytosis, and numerous Sternberg-Reed cells were characteristic of the granuloma In Case 14 remissions of 18 months followed each of two courses of nitrogen mustard The patient's course has been described in greater deail in another publication [13]

Figure 17-15 from Case 15 is an example of a typical granuloma There was marked pleomorphism and scarring (Fig 17-15*A*), but the node had many areas in which lymphocytes were numerous (Fig 17-15*B*) This biopsy was taken approximately two years after diagnosis and initiation of x-ray therapy. The patient responded well to further treatment with both x-ray and nitrogen mustard for another three years

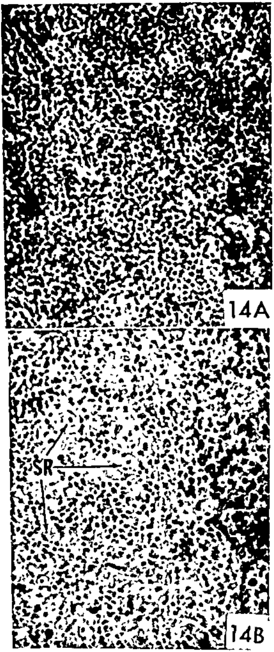


FIG 17 14 *Case 14*, Hodgkin s granuloma (*A*) Lymph node very rich in small lymphocytes, similar to a paragranuloma (× 160) (*B*) Same node as Fig 17 15*A* Another area more similar to a granuloma with fewer lymphocytes and more prominent reticulum and Reed Sternberg cells (*SR*) (× 160)

been the presence of many small lymphocytes, one of the most radiation-sensitive cells in the hematopoietic tissues.[15]

The consensus is that a Hodgkin's paragranuloma indicates a survival of 8 to 20 years in the great majority of cases. During most of this time, the patients are essentially asymptomatic and are able to live a normal and even active physical life. Most of the patients reported have been treated solely on the basis of the diagnosis per se or of the presence of a palpable node; activity as herein defined has not been the indication for therapy. Occasionally, for various reasons some patients have remained untreated. Wright described two such cases[60] In one patient who was asymptomatic, recurrence of a node occurred 8 years after biopsy. The second patient was treated initially 7 years after the diagnosis was made, purely because of palpable nodes, at the time he was asymptomatic Harrison's two untreated cases were still asymptomatic at 8 and 12 years after biopsy[26] These four cases represent as good results as are reported in treated patients The possible deleterious effect of cytocidal therapy has recently been emphasized in the German literature in which the transformation of a benign Hodgkin's granuloma to a treatment-resistant Hodgkin's sarcoma has been ascribed to therapy.[32, 61]

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Smetana and Cohen[49] and Jackson and Parker[31] described the presence of sarcoidlike lesions. These occurred in about 10 per cent of cases in my experience and have had no discernible effect on prognosis or response to therapy The major problem in this variety of Hodgkin's granuloma has been the difficulty in differentiating between it and sarcoid and other noncaseating granulomas.

Case 16 originally had a Hodgkin's granuloma. She responded favorably to courses of nitrogen mustard and x-ray therapy for five years The lymph node illustrated in Figure 17-16, obtained a few months prior to her death at the onset of a treatment-resistant stage of the disease, had undergone metamorphosis to a Hodgkin's sarcoma. The tissue was somewhat acellular and fibrous (Fig. 17-16*A*). The predominant cell was a reticulum cell with an anaplastic appearance due to a prominent nucleolus and lobing of the nucleus (Fig 17-16*B*) Lymphocytes were sparse

The following interpretations of the histopathology of Hodgkin's granuloma seem warranted and are in agreement with Jelliffe's concepts[34] The more closely the histopathology of Hodgkin's granuloma resembled that of the paragranuloma, the better was the prognosis and response to therapy. The most critical feature in this resemblance was the presence of large numbers of small lymphocytes The prognosis and response to therapy become poorer as the ratio of reticulum cells and Sternberg-Reed cells to small lymphocytes increased, that is, as the histopathology more closely resembled the sarcoma.[23, 31, 32, 35, 38] There were undoubtedly other important factors, but they were not discernible by microscopic examination

Hodgkin's Sarcoma. Patients with this entity usually presented with systemic manifestations such as chills, fever, weight loss, and anorexia The overwhelming majority have died within two years,[38] in some series all patients have died within a year[25, 49] A few patients have had a short remission following treatment, but most patients were treatment-resistant[30, 33, 37] The histopathology was the same regardless of whether the patient's disease began as a paragranuloma or granuloma or had the characteristics of a sarcoma from the onset (Fig 17-16*A*, *B*)

Figure 17-17 is from a patient who had a brief and poor response to nitrogen mustard, dying within a few weeks of treatment There was complete loss of normal architectural pattern The dominant cell was a hypertrophied reticulum cell (Fig 17-17) Occasional lymphocytes and neutrophils were scattered between the reticulum cells Mitoses and degenerating cells were frequent There was no fibrosis or eosinophilia

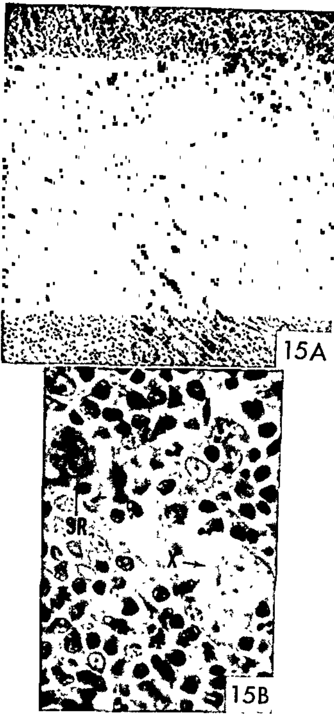


Fig 17-15 *Case 15*, Hodgkin's granuloma (*A*) Dense fibrous tissue at border of moderately cellular lympho granulomatous tissue (× 160) (*B*) Another area of same node, rich in small lymphocytes and Reed-Sternberg cells (*SR*), one of which is in mitosis (*X*) (× 650)

EVALUATION OF PROGNOSIS AND RESPONSE TO THERAPY BASED UPON EXTRANODAL TISSUES

The great majority of investigations on Hodgkin's disease have been based upon studies of lymph node biopsies because of the availability of this tissue for study without any risk and with a minimum of discomfort to the patient However, Hodgkin's disease at autopsy affects a wide variety of tissues, especially the spleen, liver, and marrow. Probably if these tissues were more

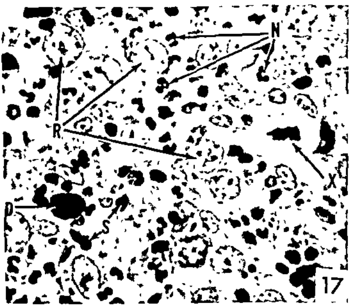


FIG 17-17 *Case 17*, Hodgkin's sarcoma (reticulum cell sarcoma?), lymph node Dominant cell is a reticulum cell (*R*), occasional small lymphocytes (*S*), and neutrophils (*N*), many mitoses (*X*), and degenerating cells (*D*) (× 900)

readily accessible for microscopic study, there would be fewer investigators who believe in the localized origin of Hodgkin's disease

Liver. There are no systematic studies available on the liver in Hodgkin's disease except for those obtained at autopsy My own material consists of fragmentary and irregular observations[9, 10, 13, 14] Early in the disease the morphologic feature were nonspecific, consisting of a minor degree of portal infiltration and hyperplasia of Kupffer cells Most of the biopsies were obtained from patients who were seen as diagnostic problems, usually presenting with fever of unknown origin, such patients are identified with a poor prognosis It has been impossible to evaluate liver biopsy as an index

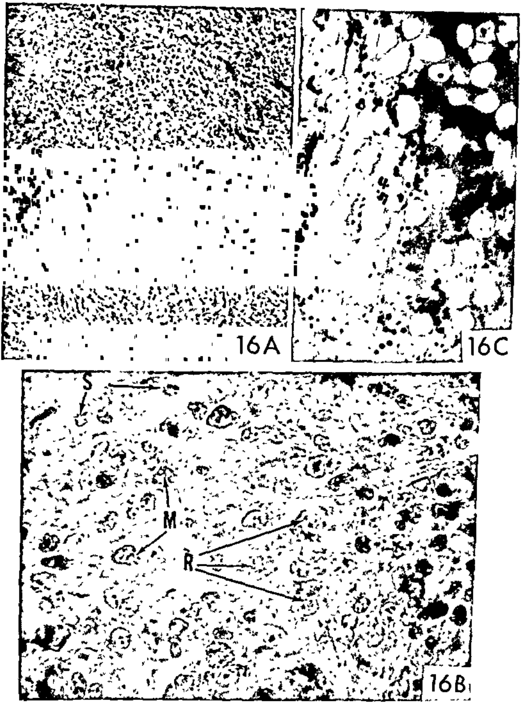


FIG 17-16 *Case 16.* Hodgkin's granuloma after metamorphosis to Hodgkin's sarcoma (*A*) Lymph node, loss of architectural pattern, moderately acellular (× 160) (*B*) Predominant cell is a hypertrophied reticulum cell (*R*), small (*S*) and medium sized (*M*) lymphocytes very sparse, same node as Fig 17 17*A* (× 650) (*C*) Marrow, extreme serous fat atrophy (× 80)

at autopsy.[50] He found marrow affected in 78 per cent of cases but implied that if enough areas of active marrow are studied, the specific lesion should be demonstrable in all patients. Depending upon the method of study, Hodgkin's tissue was recognizable in from 0 per cent to 15 per cent of cases studied prior to autopsy. Pettet and colleagues demonstrated the lesion in 9 of 31 cases.[43] Five other cases had nonspecific lesions which were abnormal but not pathognomonic of Hodgkin's disease.

My own experience[9, 14] with at least a hundred cases, many of which had multiple biopsies, was at variance with both Steiner and Pettet. A lesion pathognomonic of Hodgkin's has been demonstrated in about 10 per cent of cases (Fig 17-18). Case 18 was typical of those in which a specific lesion was demonstrable in the marrow. The patient presented with fever of unknown origin and with a pancytopenic blood picture The lesion in such instances has usually been a sarcoma or intermediate between a sarcoma and granuloma and has almost invariably been correlated with a poor prognosis and no response to therapy (Fig. 17-18*B*). Pettet, Rappaport, and their colleagues also noted that involvement of the marrow was seen in patients with far-advanced disease (and presumably a poor prognosis) or patients with pancytopenia.[43, 45] The poor prognosis in these cases was correlated with the histopathologic characteristics of the specific Hodgkin's lesion rather than with the extent of marrow replacement (Fig. 17-18). Even at autopsy, physical replacement of the marrow was a very rare occurrence.[50]

The second facet, the amount and character of the residual myeloid tissue, was more frequently a guide to therapy than was the comparatively rarely demonstrable specific Hodgkin's lesion The more benign the prognosis and/or the earlier in the stage of the disease that the patient is encountered, the more granulocytic and megakaryocytic hyperplasia there was Figure 17-13*C* is from a marrow biopsy obtained from Case 13 in 1953, immediately prior to a three-year remission induced by nitrogen mustard. A patient with so hyperplastic a marrow will not develop severe pancytopenia following either nitrogen mustard or x-ray therapy and will usually obtain a remission

Over the course of years, as patients lost weight, the marrow underwent a serous rather than a simple fat atrophy This change is illustrated by Figure 17-13*D* obtained from Case 13 in 1958 when the patient had begun to lose weight At that time he developed mild pancytopenia, and subsequent courses of therapy have produced remissions measured in months and not years The marrow had undergone a moderate serous fat atrophy, but there still was enough myeloid tissue to withstand the effects of a course of nitrogen mustard

Figure 17-16*C* was obtained from Case 16 a few weeks prior to death when the patient was severely pancytopenic, febrile, and emaciated She had al-

to prognosis and therapy because of the lack of a sufficient range of material, i.e., benign as well as malignant types of Hodgkin's disease early in its course. However, it is common experience in Hodgkin's disease that, when there has been marked hepatic involvement, the prognosis and response to treatment is poor.[37]

Spleen. Spleen biopsies have been even more dangerous to do than liver biopsies, but more studies are available than in the case of the liver because of the more obvious involvement of the spleen early in the natural history of Hodgkin's disease.[9, 10, 13, 14] Although, in general, the disease in the spleen paralleled that in the lymph nodes, it was not uncommon for splenic tissue to be uninvolved by Hodgkin's disease in a patient in whom node biopsy has been positive. Biopsy of the spleen offered little additional information over that obtainable from study of a lymph node removed concomitantly. Response to therapy and prognosis were governed by the concentration of lymphocytes The more lymphocytes or, in other words, the closer the resemblance to Hodgkin's paragranuloma, the better has been the response to therapy and the better the prognosis. In addition one may assume that following therapy an intensely fibrotic spleen will not shrink as much as one with less fibrosis

Marrow. Of all the tissues involved in Hodgkin's disease, the marrow has been studied the least. The reasons are as follows. First, most pathologists consider Hodgkin's disease to be a disease of lymphatic tissue and consequently believe there is little merit in the study of myeloid tissue despite Steiner's report which emphasized the frequency of marrow involvement.[50] Second, analysis of smears of aspirated marrow has been the usual method employed by the hematologist for studying marrow Although this technique may suffice when there is a qualitative change in the marrow, it is not adequate for evaluation of the much more subtle changes in Hodgkin's disease [8] For example, Cooper and Watkins[18] in 1949 concluded on the basis of smears of aspirated marrow that this tissue was of little use in evaluation of patients with Hodgkin's disease In 1950, Cooper and colleagues began the study of sections of marrow and came to the opposite conclusion.[43]

There are two areas for evaluation in the study of the marrow in Hodgkin's disease Hodgkin's tissue itself and the residual nonmalignant myeloid tissue The former was of lesser importance in the majority of cases Not enough attention has been paid to the latter for reasons already mentioned

Demonstration of Hodgkin's tissue in the marrow is dependent upon the number of lesions which in turn determines the likelihood with which the lesion may be demonstrated by biopsy The incidence and extent of involvement have been reviewed by Steiner, based primarily upon marrow studied

ready proved resistant to therapy. The severe serous fat atrophy was not pathognomonic of Hodgkin's disease but has been seen in a wide variety of diseases which have had as their common denominator long-standing progressive weight loss. Serous fat atrophy has been produced by protein deficient diets in animals,[2] by malnutrition in man,[34] by total body irradiation,[35] by Aminopterin, and by nitrogen mustard.[14] After treatment of a patient with such a lesion by nitrogen mustard, and to a lesser extent by local x-ray therapy, severe, even fatal pancytopenia often ensued. Furthermore, patients with this type of marrow lesion were prone to develop active tuberculosis or disseminated mycosis, especially after systemic cytocidal therapy. Therefore, the major importance of bone marrow histopathology (provided adequate sections are studied) was that it provided assessment of the patient's ability to withstand the cytocidal effect of systemic chemotherapeutic agents such as nitrogen mustard

Peripheral Blood. Since the peripheral blood reflects the marrow, one may obtain invaluable information from the determination of the absolute number of neutrophils and platelets When an exacerbation of Hodgkin's was accompanied by a polynucleosis and thrombocythemia, the marrow was usually hyperplastic There were two exceptions: first, the occasional patient with a benign or treatment-responsive disease in whom an exacerbation was accompanied by neutropenia even though the marrow showed myeloid hyperplasia and, second, the splenectomized patient who may have a polynucleosis and adequate platelets in spite of an atrophic marrow. Treatment of such a patient with systemic agents has been disastrous, producing fatal pancytopenia

Significance of Fibrosis

It was very difficult to evaluate the significance of fibrosis in a node biopsy in terms of prognosis and response to therapy Once a biopsy had been taken of a node so that the degree of fibrosis could be estimated, further observations upon that node were manifestly impossible. Nevertheless, Smetana and Lumb have concluded that sclerosis carried a favorable prognosis.[37, 49] The evidence for this statement was not unequivocal To complicate the problem, both Hodgkin's paragranuloma and Hodgkin's sarcoma, representing respectively the forms with the best and poorest response to therapy, were characterized by a minimum of fibrosis

Splenic tissues offer more information, by repeated sampling with an adequate technique the spleen may serve as its own control[9, 10, 13, 14] A markedly fibrotic spleen did not shrink under therapy as much as did a less fibrotic spleen In general, but not invariably, diminution in size under therapy was correlated with a good clinical response It is my view based on

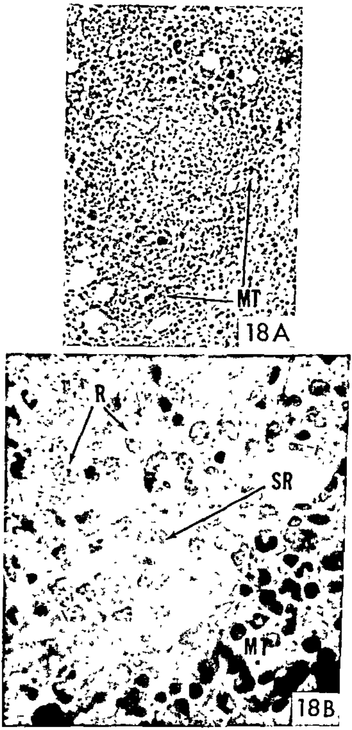


Fig 17 18 *Case 18*, Hodgkin's sarcoma (*A*) Bone marrow, nodule of Hodgkin's sarcoma surrounded by moderately hyperplastic myeloid tissue (*MT*) (× 80) (*B*) Same nodule as *A*, composed primarily of reticulum cells (*R*) and Reed Sternberg cells (*SR*), bordered by hyperplastic myeloid tissue (*MT*). (× 650)

either as Hodgkin's sarcoma or reticulum cell sarcoma.

Systemic symptoms, especially malaise, fever, weight loss, and anorexia, predominated in patients with reticulum cell sarcoma. The overwhelming majority of patients have died within two years of onset.[31, 32, 37, 39, 61] As in Hodgkin's sarcoma, there was very little evidence that a significant remission has ever been induced in any patient.[30, 32]

DISCUSSION

Histopathologic analysis should not only serve as a means of diagnosis but should be a guide to prognosis and therapy. This utopian objective will probably never be attainable solely on the basis of morphology, even if the present-day custom of relying on a single biopsy is changed to one of analysis of multiple biopsies. Consequently, the microscopist must not adopt too narrow an attitude but must take into account other aspects of the patient's illness such as the presenting symptoms, location of tumors, the presence of abnormal proteins, and the type and amount of treatment.[41, 47] In other words, in the present state of our knowledge, histopathologic analysis must be supplemented by a thorough acquaintance with all aspects of the patient's disease.

Histopathologic Analysis

In order to obtain a maximum amount of information from morphology, one must consider the dominant cell type, architectural pattern, cellularity of the marrow, and the changing morphologic pattern.

Dominant Cell Type

The dominant cell type was probably the most important single factor. When the microscopic field was usurped almost exclusively by small lymphocytes, the prognosis was the most favorable within any specific architectural pattern. For example, Hodgkin's paragranuloma, Type I giant follicular lymphoma of Gall and Mallory, and lymphocytic lymphosarcoma represented the most benign varieties of Hodgkin's disease, giant follicular lymphoma, and lymphosarcoma, respectively. Patients with these forms were the ones who responded best to therapy. They also were the patients who least needed treatment, if one accepts the indication for treatment outlined in this chapter. As the dominant cell type approached the reticulum cell morphologically, the prognosis and response to therapy became poorer.

Between the two extremes of cytologic differentiation, there were an infinite number of gradations which we have subdivided for each architectural pattern as a matter of clinical convenience. These gradations are beautifully illustrated in the semidiagrammatic figure in the paper by Warren and

admittedly fragmentary observation of spleen biopsies, which are nevertheless probably more valid than those made on nodes, that the amount of fibrosis tended to be inversely correlated with the clinical response.

Histopathology as a Guide to Therapy in Hodgkin's Disease

There were two aspects to be considered: when to treat a patient and which therapeutic agents should be utilized. For years the former problem was simple—the indication for treatment was a diagnosis of Hodgkin's disease based on biopsy. This attitude is no longer valid if one subscribes to the concept that treatment should be reserved for the patient with active disease as *defined in this chapter. Histopathology would then be of lesser* importance than a weight scale and a thermometer in deciding when to initiate treatment. As already mentioned, study of the marrow was an excellent method for determining when not to treat a patient.

Our choice of treatment is limited to two types, systemic and local. Systemic therapy is synonomous with nitrogen mustard or any of the closely related compounds such as urethane, chlorambucil, and TEM. Despite the enthusiasm engendered by each investigator who introduced a variant of the original nitrogen mustard, all these compounds are similar in mechanism of action and therapeutic index.[34, 51, 52] Consequently, analysis of biopsy tissue can hardly play a role in a selection of one type of chemotherapy in preference to another. However, local x-ray therapy, since it has little if any effect on marrow not directly exposed to the x-ray beam, has caused a lesser degree of pancytopenia than nitrogen mustard. Consequently, x-ray therapy has been safely used when nitrogen mustard is contraindicated because of marrow depletion (Figs. 17-13*D*, 17-16*C*). However, x-ray therapy under these circumstances may not induce a remission.

There was no histologic pattern correlated with a response to nitrogen mustard as opposed to x-ray therapy or vice versa. The decision to use one or the other is based more upon whether a patient has systemic or local manifestations of activity.

Reticulum Cell Sarcoma

The separation of reticulum cell sarcoma from Hodgkin's sarcoma has been difficult, some authorities held that they are basically similar.[32, 61] In general, if a patient has been known not to have had Hodgkin's disease and if Sternberg-Reed cells were not demonstrable, the diagnosis of reticulum cell sarcoma rather than of Hodgkin's sarcoma has been made. However, the distinction *between an anaplastic reticulum cell with a hypertrophied* lobate nucleus and a developing Sternberg-Reed cell may be difficult and perhaps invalid. Figure 17-17 illustrates a biopsy that could be classified

there any evidence, once significant interference with marrow function had occurred by virtue of replacement by lymphatic tissue, that cytocidal therapy induced a regeneration of myeloid tissue even if the amount of lymphatic tissue decreased Rather, severe serous fat atrophy leading to disability and death from pancytopenia developed (Fig. 17-11) As a result evaluation of marrow histology with respect to the adequacy of the myeloid tissue was an important aspect in management. Progressively extensive replacement of myeloid tissue indicated a decreasingly poorer response to therapy and a poorer prognosis Progressive serous atrophy, part of the natural history of the diseases which was aggravated by treatment, has had a similar significance.

Since the introduction of radiation and chemotherapy, the "laws" of Bergonié and Tribondeau have dominated our thinking.[13] These authors originated the concept that immature, rapidly dividing cells were most sensitive to irradiation Examination of their data indicated that their laws were based upon observations made on the testis of one rabbit.[14] On the other hand, starting with Heineke's[24] experiments, there have been numerous studies to indicate that these laws are invalid [12, 13, 15] Similarly, paralleling our faith in these laws, but again lacking any verification, there has been tacit acceptance of the belief that malignant cells were more sensitive to irradiation than corresponding nonmalignant cells. However, serial biopsy of tissues of patients with malignant diseases of the hematopoietic tissues has effectively disproved this concept [13, 28] Case 9 illustrated what happened to the marrow when an attempt was made to "burn out" the malignancy. One must anticipate damage to myeloid tissues as well as to the lymphatic tissues when a patient with a lymphatic malignancy is treated with isotopes or with chemotherapy The degree of anemia, thrombopenia, or neutropenia following treatment depended on the amount of myeloid tissues present prior to treatment and the dose and duration of treatment [9, 14] As the amount of myeloid tissue decreased, whether because of atrophy or replacement, the patient was more likely to develop pancytopenia of serious degree following treatment Conversely, the more active the myeloid tissue, the more likely the patient was to withstand the cytocidal effect of treatment and to obain a remission

The situation became more complicated because hyperplasia was often an evidence of a complicating factor such as abnormal destruction of red cells, white cells, and platelets Frequently the hypersplenic syndrome was a more serious problem than other aspects of the lymphomatous disease Evaluation of the role of the marrow is predicated upon the availability of sections of a suitable biopsy specimen Smears of aspirated marrow were unreliable for the purpose outlined

With some practice one may estimate the adequacy of the marrow from

Picena.[75] At the risk of oversimplification, one might say that the larger the dominant cell and the more delicate its standing characteristics, the poorer the prognosis and the poorer the response to therapy.

Architectural Pattern

The architectural pattern of the malignant tissue ranked next to cytology in importance. In general, the better the preservation of architecture, the better was the prognosis.[7, 59, 60] Rappaport and colleagues' study in particular indicated that preservation of follicular pattern was a better prognostic sign than is loss of normal architecture.[45]

Fibrosis

The role of fibrosis was difficult to evaluate. Some authors believed that it has been associated with a relatively good prognosis, although the evidence upon which this content was based was meager.[37, 49] My own observations, based primarily upon serial splenic biopsies, indicated that extensive fibrosis was correlated with a lack of diminution of size of involved tissues after any type of therapy, and with a poor prognosis as well. In the individual patient there was progressive fibrosis during the natural history of that patient's disease, and in this sense fibrosis was correlated with an increasingly poorer response to therapy.[13, 14]

Special Role of the Marrow

Bone marrow has played a much neglected role in the evaluation of prognosis, natural history, and feasibility of, and response to, therapy. Since the introduction of radiotherapy, and especially in the more recent chemotherapeutic era of the last 15 years, we have become mesmerized by the guileless concept that destruction of the cell type characteristic of the malignancy is the goal of treatment Increasingly less attention has been paid to an extremely important aspect, the ability of the marrow to produce red cells, white cells, and platelets in normal numbers under conditions that will insure normal longevity of these formed elements in the circulating blood In addition, in the presence of hemorrhage or infection, the marrow must be able to produce an increased number of these elements. Another function that was correlated with prognosis was that of the production of an adequate antibody response when the need arose Observation of patients with lymphatic malignancies cannot help but impress the physician with the importance of anemia, thrombocytopenia, neutropenia, and poor antibody response as major causes of disability and death

Conversely, there was no proof that an increase in lymphocytes in the peripheral blood or marrow, except when they grossly replaced myeloid tissue, had any real significance as a cause of disability or death Nor was

there any evidence, once significant interference with marrow function had occurred by virtue of replacement by lymphatic tissue, that cytocidal therapy induced a regeneration of myeloid tissue even if the amount of lymphatic tissue decreased. Rather, severe serous fat atrophy leading to disability and death from pancytopenia developed (Fig 17-11) As a result evaluation of marrow histology with respect to the adequacy of the myeloid tissue was an important aspect in management. Progressively extensive replacement of myeloid tissue indicated a decreasingly poorer response to therapy and a poorer prognosis Progressive serous atrophy, part of the natural history of the diseases which was aggravated by treatment, has had a similar significance.

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With some practice one may estimate the adequacy of the marrow from

the blood smear. If platelets and neutrophils were not present in sufficient numbers, the marrow biopsy will determine if this depression is due to atrophy, invariably serous and not simple, or due to a hypersplenic syndrome.

Changing Morphology

Most studies on the lymphocytic malignancies have laid emphasis upon the progressive nature of these diseases,[31, 35, 36] with inevitable metamorphoses toward a more malignant, less treatment-sensitive condition. Accordingly, one would expect that the histopathology of the lesions in a given patient would also change; as a result serial biopsies would be needed to keep pace with these changes.

The diseases under consideration were notoriously accompanied by various complications. Hypersplenic syndromes, acid fast, fungal, and pyogenic infections, abnormal proteins, and poor antibody formation were examples In addition the patient may have had coincidental disease, such as occurred in Case 6 of this report and in Walter's case,[56] the manifestations of which were erroneously ascribed to the lymphoma Serial biopsy was needed to determine whether failure to respond to therapy in a previously responsive patient was due to a change in the lymphoma to a more malignant type, or whether the lymphoma had responded but the persistent symptoms were due to a complicating factor. If a patient who has had a paragranuloma failed to respond to therapy at a time when a repeat node biopsy had shown a change to Hodgkin's sarcoma, the therapist may reasonably ascribe the failure to a change in the biologic nature of the disease However, if the node biopsy in this patient still was characteristic of a paragranuloma or granuloma, then the physician should logically search for some complicating disease An excellent example recently encountered was that of a man with benign Hodgkin's disease which was treated repeatedly with nitrogen mustard, soft skin x-ray, and prednisone for pruritus Thereafter, the patient developed severe chills and fever, mistakenly ascribed to the Hodgkin's disease At autopsy these symptoms were found to be due to systemic nocardiois.

Ancillary Factors

Sites of Involvement

The sites of demonstrable involvement of disease have had an undoubted bearing upon prognosis and response to therapy Unfortunately, this aspect of the problem has been beclouded by limitations in our ability to recognize areas of involvement other than those obvious by the ordinary tech

niques of physical, x-ray, and laboratory examination For example, Hodgkin's paragranuloma has been considered to be limited to a single area merely because no other masses were demonstrable. This naive approach to the problem obviously had no validity in excluding the presence of asymptomatic areas of microscopic and even gross involvement by the disease in the more inaccessible parts of the patient's body. One of my patients with Hodgkin's disease was killed in an accident twenty-four hours after posteroanterior and lateral x-ray films of the chest were read as negative for mediastinal involvement. At autopsy walnut sized nodes were found scattered through the entire mediastinum.

There were certain anatomic areas of involvement that were inherently associated with a poor prognosis Under such circumstances a patient with a biologically benign type of disease, on histopathologic grounds, may have a lesion which in itself caused such a serious train of complications that it induced an unfavorable or lethal clinical complex. The most striking example was a patient referred for treatment with a benign Hodgkin's paragranuloma. He had developed lumbar transverse myelitis due to an unrecognized lesion in the dura Death resulted from renal tract infection, stones, decubital ulcers, and inanition rather than from the Hodgkin's disease itself.

Extension of the lymphoma from the mediastinum into the lung parenchyma foreboded a poor prognosis.[11, 41] This type of lesion interfered with pulmonary drainage and caused recurrent intractable pulmonary infections. X-ray therapy may give temporary relief, but the resulting fibrosis further decreases pulmonary drainage. The majority of patients died within one year of the time that this complication was clinically apparent. For unknown reasons patients with chronic lymphatic leukemia have had a relatively better prognosis with this complication

Occasionally a patient has developed severe, intractable pruritus in the course of what would otherwise have been a comparatively benign disease. These patients literally scratched their way into the grave and died of a combination of inanition and infection, abetted by the complications which followed large doses of steroids and chemotherapy Severe liver disease has resulted in intractable ascites or obstructive jaundice with ascending biliary tree infection

If the lymphoma is first manifested in the stomach,[23] orbit, or nasopharynx, the prognosis may be unusually benign [14]

LABORATORY FINDINGS

There were several complications demonstrable by laboratory study that grossly modified the interpretation based upon histopathology alone. Severe anemia, neutropenia, and thrombopenia out of line with the pathologic

process were the best examples of this phenomenon. Abnormal proteins also modified the patient's prognosis and response to therapy to an extent which invalidated an interpretation based purely upon microscopic analysis. These rather rare complications often did not respond to therapy, even when all other aspects of the patient's disease had been brought under control.

Pre-existing or Coincidental Disease

In this category, which modified histopathologic considerations, we must consider two possibilities, intercurrent disease that has no relationship to the lymphoma and intercurrent disease that tends to occur or undergo exacerbation as a result of the poor resistance of the patient. The former group was obvious, typified by the lack of tolerance of severe anemia by a patient who had severe cardiac insufficiency.

The latter category was much commoner. Patients with lymphomas are notoriously susceptible to infection, especially tuberculosis and mycotic disease. Chemotherapy, steroids, and, to a lesser extent, x-ray therapy further reduced the host's immunologic and cytologic defenses against infection Recognition of a complicating infection was often difficult since the symptoms may closely simulate those of activity of the lymphoma. Serial biopsy has fortuitously unveiled the complicating infection.

Prior Therapy

Overzealous treatment was one of the most potent factors in inducing a poorer prognosis, it served as a cause for a serous atrophy of the marrow sufficient to make further therapy dangerous Serous atrophy of fat was especially prone to follow chemotherapy and radioisotope therapy because these agents exerted a systemic effect which depleted the marrow Local x-ray therapy was much less dangerous since only the marrow in the path of the x-ray beam was significantly affected Steroids were not toxic to myeloid tissue and so were not hazardous in this regard

Case 6 was an excellent example in which histopathologic analysis indicated that a patient with chronic lymphatic leukemia had a relatively benign disease and did not therefore require treatment Unfortunately, her marrow was so badly injured by over treatment that it led to her death from pancytopenia

CONCLUSIONS

The term "favorable response to therapy" was limited to those situations in which a patient, previously physically unable to meet the stress of a normal way of life, was thereafter able as a result of treatment to cope physically with these demands

The most important morphologic factors in evaluating prognosis and response to therapy were:

1. The dominant cell type
2. Functional capacity of the marrow
3. Changing histopathology
4. Preservation of architectural pattern
5. Development of fibrosis

Ancillary data modifying conclusion based upon histopathology were:

1. Sites of involvement by malignant tissue
2. Presence of coincidental disease
3. Lowering of resistance to infection
4. Presence of hypersplenic syndromes or abnormal proteins
5. Overtreatment.

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Case 6 was an excellent example in which histopathologic analysis indicated that a patient with chronic lymphatic leukemia had a relatively benign disease and did not therefore require treatment Unfortunately, her marrow was so badly injured by over treatment that it led to her death from pancytopenia.

CONCLUSIONS

The term "favorable response to therapy" was limited to those situations in which a patient, previously physically unable to meet the stress of a normal way of life, was thereafter able as a result of treatment to cope physically with these demands

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CHAPTER 18

LYMPHOPROLIFERATIVE DISORDERS

WILLIAM DAMESHEK

The lymphoproliferative disorders include both isolated and generalized proliferations of lymphoid tissue. The generalized proliferations may be benign, reactive, and self-limited—as in certain infectious disorders and in the primary immune response. The localized proliferations of lymphoid tissue are neoplastic, although their degree of malignancy varies greatly In varying lengths of time, they tend to become generalized There is little if any distinction between the generalized lymphosarcomatous processes and lymphocytic leukemia; histologically, they are indistinguishable

Chronic lymphocytic leukemia is perhaps the commonest of the forms of leukemia in the West. There are "aggressive" types with considerable lymphadenopathy, splenomegaly, and systemic manifestions There are "benign" types without lymphadenopathy or splenomegaly and completely asymptomatic Autoimmune hemolytic anemia occurs in both these types and is often by far the important feature of the leukemic process Hyperglobulinemia and hypogammaglobulinemia may be found.

Macroglobulinemia may occur in occasional cases of generalized lymphocytic proliferation and may sometimes be the outstanding manifestation. With a considerable quantity of heavy globulin, hemorrhagic disease may ensue and numerous infections may develop, presumably—as in multiple myeloma—because normal antibody globulin production is diverted to the production of this abnormal globulin Waldenström's macroglobulinemia is probably best considered as a form of lymphoproliferative disease.

The lymphocytic tissues are involved in anitbody production. Thus, it is not strange that when they are proliferating in a general fashion, the produc tion of abnormal globulins is greatly increased. Some of these may be cell-specific (as against red cells, i e autoimmune hemolytic anemia), at times other manifestations are seen, including thrombocytopenic purpura of the

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matic variety of the older age group. Here treatment whether by x-rays, alkylating agents, or the corticosteroids may well do much more harm than good and may even do irreparable harm. The physician must know when *not* to treat as well as when to apply therapy. It is not necessary always to treat according to the number of cells present, but therapy should rather be used only if the patient needs it and if it will do no harm.

EDITOR'S NOTE. For a detailed discussion of this author's views, readers are referred to the recent textbook *Leukemia* by W. Dameshek and F. Gunz, New York, Grune & Stratton, Inc., 1958.

"idiopathic" type, Henoch-Schonlein purpura, and leukocytic antibodies In a benign lymphoproliferative disorder—infectious mononucleosis—there is a "heterophile" antibody against sheep red blood cells Other viral infections with lymphocytic proliferation as one feature show antibody reactions of one sort or another.

The lymphoproliferative disorders may be treated by x-ray and the alkylating agents, including nitrogen mustard, TEM, and Leukeran. These are destructive agents both to lymphoid tissue as well as to the bone marrow and must be used with considerable caution else severe cytopenias, sometimes irreversible, ensue On the other hand, ACTH and the corticosteroids are lymphocytolytic *but not myelotoxic.* When given in massive dosage in acute lymphocytic leukemia, they may induce complete remissions. In chronic lymphocytic leukemia they are often highly effective, at least temporarily, in cases that have become refractory to the destructive agents and in which bone marrow depression is present. The results with the corticosteroids in the chronic lymphoproliferative disorders, particularly when large lymphoid masses, cachexia, and severe anemia are present, are often very striking With the use of large-dose corticosteroid therapy (prednisone, 50–100 mg daily), the masses usually regress considerably, the systemic symptoms disappear, and the anemia becomes greatly improved In the cases with autoimmune hemolytic anemia and hyperglobulinemia, the results are dramatic. Experience with well over 50 cases of extensive, generalized chronic lymphoproliferative disease now indicates that large-dose corticosteriod therapy may well be the treatment of choice in many instances, not only when resistance to x-ray and alkylating drugs has deve'oped but even as the initial procedure From our detailed observations in recent studies, it seems more than likely that the corticosteroids are lymphocytolytic agents with an activity comparable to that of x-ray Long-term therapy with the corticosteroids is, of course, subject to various hazards, but alternation of these materials with the alkylating agents may be helpful in this regard

Primary lymphosarcoma of the spleen is the commonest neoplasm of the spleen At first it simply presents as splenomegaly and later as hypersplenism with cytopenias but with a normal bone marrow Eventually lymph nodes, the bone marrow, and blood are involved, giving the picture of leukemia, leukemic or aluekemic The 'moving picture" of the various events in this neoplastic disorder is of considerable interest Therapy differs according to the stage of the disease and may include splenectomy, x-rays, the alkylating agents, or the corticosteroids

No discussion of the treatment of the lymphoproliferative disorders would be complete without emphasizing the importance of *no therapy* in some cases, particularly in chronic lymphocytic leukemia of the benign, asympto-

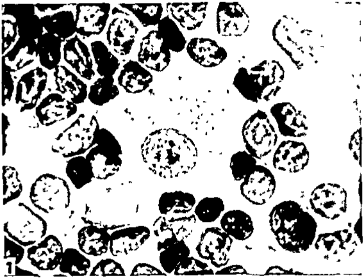


Fig 19 1 Inactive reticulum cell (*center*) surrounded by small and medium sized adult lymphocytes Lymph node imprint in cat scratch disease. Leishman stain (× 1100)

Table 19 1. Malignant Lymphomas

RETICULUM CELLS
1. Reticulum cell sarcoma*
2. Hodgkin's disease*
3. Letterer Siwe's disease
4. Ewing's tumor?

↓

HEMATOPOIETIC RETICULOENDOTHELIAL CELLS
1. Acute "monocytic" leukemia

↙ ↘

LYMPHOBLAST
1. Acute lymphocytic leukemia
2. Lymphosarcoma*

↓

PROLYMPHOCYTE
1. Subacute lymphocytic leukemia
2. Lymphosarcoma*

↓

LYMPHOCYTE (large, medium, small) —— 1 Waldenstrom's syndrome ——→ PLASMA CELL
1. Chronic lymphocytic leukemia
2. Lymphosarcoma*

PLASMABLAST
1. Multiple myeloma
2. Plasmacytic leukemia
3. Solitary plasmacytoma

↓

PROPLASMACYTE

↓

PLASMA CELL

* *The nodular manifestation of each of these diseases is giant follicular lymphoma.*

CHAPTER 19

STRUCTURE OF THE LYMPHOCYTIC SERIES OF CELLS IN RELATION TO DISEASE

JOHN W. REBUCK

The histologic appearance of the malignant lymphomas and related lymphocytic leukemias as seen in sections is now familiar to American pathologists through the detailed reports of Warren and Picena,[60] Gall and Mallory,[25] Custer,[9] Richter,[50] and Berman.[2, 3] The reader is urged to familiarize himself with these works. Readily available is the text by Gall and Rappaport[26] on diseases of the lymph nodes and spleen which summarizes in full the necessary characteristics for assay of histologic sections of diseased lymphocytic tissues and at the same time brings home a growing conformity of American pathologists' attitudes toward classification of diseases of these same tissues.

Table 19-1 outlines the origins of the lymphocytic series of cells and the diseases pertaining to each cell type Cell types are designated in accordance with Standard Nomenclature[7] whenever possible Such a straightforward classification must be modified to include two further reports which merit our studied consideration A vast experience with lesions of this type during the second World War led Custer and Bernhard[10] to stress a not uncommon interrelationship among cell types of the malignant lymphoma group leading to the recognition of additional mixed types of malignant lymphomas The mixed types, then, are a spectrum of any of the cell types in Table 19-1, reading up or down the lineages Of equal importance was the re-evaluation of the follicular lymphomas by Rappaport, Winter, and Hicks,[44] demonstrating that the cell patterns of the nodular growths could be almost pure collections of any one of the five elements designated by

functions: it can be free or in syncytium, it can shed its cytoplasm in antibody formation as shown by Sabin,[52] it can become more basophilic and blastlike (Figs. 19-7 through 19-10) to become an hematopoietic reticulum cell and thus form lymphoblasts and the entire lymphocytic series, or it can become immediately phagocytic if need be and function as a fixed or free macrophage

RETICULUM CELLS

In Table 19-2 are listed some of the more important diseases affecting the reticulum cell as a reticulum cell or affecting the monocytes and macrophages which it is capable of forming In Figure 19-1 the inactive mother cell of the lymphocytic tissues is shown with its large cell body measuring from 15 to 25 μ in diameter. The abundant cytoplasm of the inactive reticulum cell may be pale to dark blue in color, and it may be homogeneous or mottled with abundant, colorless, or slightly acidophilic hyaloplasm. The cytoplasmic to nuclear ratio favors the cytoplasm Fine or large azurophilic granules may or may not be present Its nucleus may be round, oval, or reniform, but rarely is it indented The nuclear membrane is moderately thin, and the inactive reticular nucleus is small in size The chromatin pattern of the nucleus is composed of an irregular network or stipple of violet strands or granules; the interstices are irregular, angular, and unequal in size and are filled with a small amount of pink or blue parachromatin, distinct from the chromatin None or from one to six, usually from three to six, small, irregular or angular, poorly delimited, blue nucleoli can be found In spite of its inactive appearance, it is immediately phagocytic when needed The reader should consult Plate XXXI of Diggs's atlas[12] for

Table 19-2. The More Important Diseases Affecting the Reticulum Cell

RETICULUM CELL
(Mesenchymal cell)
1 Reticulum cell sarcoma
2 Hodgkin's disease
3 Letterer-Siwe's disease
4 Ewing's tumor?

→ MACROPHAGE (Histiocyte)
1. Gaucher's disease
2. Niemann-Pick's disease
3. Hand Schuller Christian disease
4. Whipple's disease

→ MONOBLAST
1 Acute monocytic leukemia (Schilling)

→ MONOCYTE
1. Chronic monocytic leukemia (Naegeli) (Myeloblast cell of origin)

an asterisk in Table 19-1 or again that the mixed type itself may be nodular or diffuse in growth pattern. We agree that lymphomas with a nodular pattern should be integrated into the general classification of malignant lymphomas by using any one of the accepted cytologic classifications for each lymphoma and adding the term "nodular" or "follicular" whenever this is indicated by the prevailing architectural pattern. In this way the prognosis as to longevity can be correlated not only as to cellular composition and cellular differentiation but also *within each cellular group* as to the presence or absence of the generally more favorable nodular or follicular pattern of growth. The reader should have access to descriptions of the histologic architecture of the lesions of Hodgkin's disease in the excellent and detailed publication of Jackson and Parker.[30] Somewhat divergent views from abroad may be examined in the texts of Lumb,[38] Marshall,[39] Moeschlin,[40] and Bessis[4] in respect to the entire field of the lymphomas and related leukemias

Usually the histologic pattern of the lesions of the lymphocytic tissues is sufficient to establish their benign or malignant nature whereas the air-dried imprints of the same lesions, stained like blood smears, are often needed for accurate identification of predominant cell types. In this supplemental fashion the use of imprints after study of the sections is advantageous and often necessary Berman[3] cautions, and it should be repeated, that imprints alone are usually inadequate for establishing whether or not a lesion is malignant unless bizarre and malignant cells are predominant because many of the individual cells seen in imprints from neoplastic lesions are also present in reactive or even normal lymphocytic tissues Downey and his associates[33, 55] were the first in this country to popularize the use of imprints of hematopoietic tissues to gain the more precise information to be obtained by inspection of air-dried films, information which is so readily correlated with the great amount of information already accumulated for bone marrow and blood cells by classic hematologic methods There is a growing literature on the utilization of the imprint technic as an adjunct in the study of lymphocytogenesis and neoplasms of lymphocytic tissues[2, 3, 33-35, 40-43, 46, 53-55, 57-59]

It is the purpose of this chapter to describe the principal cells of the lymphocytic tissues as seen in air-dried preparations of imprints or smears and the disturbances to which they fall prey As far as is applicable, recommendations of the committee for clarification of nomenclature of the blood and blood-forming organs will be utilized[7] In 1912 Downey and Weidenreich[18] crystallized evidence that the reticuloendothelial (RE) cells (reticulum cells, mesenchymal cells, histiocytic system of cells) were the mother tissue cells for the lymphocytes The inactive RE cell depicted in Figure 19-1 has many

two conditions The lipid presents in a globular inclusion in Niemann-Pick's disease, but it is noteworthy that the inclusions in Gaucher's disease are in the form of colorless spindles throwing the unaffected basophilic cytoplasmic areas into blue fibrillar-like arrangements. Cavanagh[6] has recently summarized the available knowledge of the chemistry, cytology, and histogenesis of these and related conditions In this connection, imprint studies of the affected cells in Letterer-Siwe's disease and eosinophilic granuloma, conditions somewhat related to Hand Schuller-Christian disease, should afford greater insight into the true nature of these conditions.



3

FIG. 19 3 Phagocytic reticulum cell, the lipid histiocyte of Hand-Schuller Christian disease Bone marrow. Leishman stain (× 1100)

We have imprinted a single case of each Letterer-Siwe's disease and eosinophilic granuloma of bone. In the former, rather uniform mononuclear, inactive-appearing histiocytes apparently of reticulum cell origin with pale, homogeneous cell bodies were found in company with giant, polymorphonuclear or multinucleated pleomorphic malignant-appearing reticulum cells In the latter, eosinophilic granuloma of bone, again uniform, mononuclear, inactive-appearing histiocytes very similar to those seen in Letterer-Siwe's disease were numerous, but the malignant-appearing giant reticulum cells were not present and in their place were numerous eosinophilic granulocytes.

Latest member of this group with accumulation of characteristic sickle-form PAS-positive cytoplasmic particles in the reticulum cells, again in

excellent colored illustrations of macrophages with phagocytized *Histoplasma capsulatum* and *Leishmania donovani.* Figure 19-2 depicts a large epithelioid reticulum cell in a lymph node imprint obtained from a patient with cat-scratch disease. In forming the epithelioid cell of this disease,[11] the nucleus has become larger and the nucleolar apparatus hypertrophied, although the reticular chromatin pattern remains The cytoplasm has increased in bulk and presents large pseudopodial processes suggestive of secretory activity.

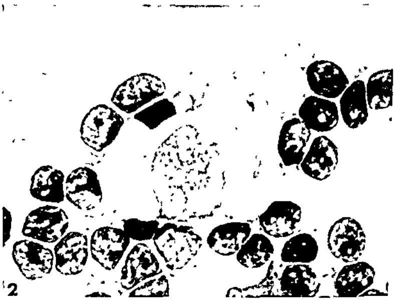


FIG. 19 2 Large epithelioid reticulum cell (*center*) surrounded by small and medium-sized normal lymphocytes Lymph node imprint in cat-scratch disease Leishman stain (× 1100)

STORAGE RETICULUM CELLS

The reticulum cells may be the site of undue accumulations of lipids and other substances resulting in multifocal proliferative diseases of the lymphocytic and connective tissues Characteristic of this group is the lipid histiocyte depicted in Figure 19-3 The nucleus, which has remained small, as in the inactive form, is largely obscured by the lipid inclusions of Hand-Schüller-Christian disease which frequently consist of large quantities of cholesterol lipids. The affected reticulum cells of Gaucher's disease containing excess cerebrosides and of Niemann-Pick's disease with excess sphingomyelin have been earlier described and depicted[46] Plate XXX of Diggs's work[12] has colored illustrations of typical lipid histiocytes in these latter

monocytic leukemia. The bizarre nuclear outlines apparent in some of the malignant reticulum cells of Figure 19-1 should not alone be misconstrued as diagnostic of monocytic differentiation. If one is to make the diagnosis of monocytic leukemia of the Schilling type, evidence for differentiation to true adult human monocytic forms (extreme right of Fig 19-10) should be established. Far more often the leukemic manifestation of reticulum cell malignancy, although similarly classified in much of the literature as



5

FIG 19 5 A lymph node imprint in Hodgkin's disease showing (*bottom*) a malignant reticulum cell, (*center*) a Reed-Sternberg cell, and (top) a spindle shaped endothelial cell Leishman stain (× 1100)

"monocytic" leukemic, is merely a leukemic outpouring of the malignant reticulum cells depicted in Figure 19-4 in considerable numbers into the patient's blood stream (Fig 19 9) Cases of this type in which the leukemic process is centered primarily in the malignant reticulum cells have been reported and correctly designated by Ewald,[20] Downey,[18] and others as leukemic reticuloendotheliosis With this background it is far more reasonable to appreciate the rare "monocytic" leukemia complicating Letterer-Siwe's disease or Hodgkin's disease as a similar accession of the malignant reticulum cells basic to these conditions to the blood stream.

Characteristic of the imprinted lesions of the second reticular malignancy, Hodgkin's disease, are the giant, pleomorphic Reed-Sternberg cells, one of which is depicted in the center of Figure 19-5 All transitions are found between this giant cell peculiar to Hodgkin's disease and the atypical, large,

multifocal proliferative condition, is Whipple's disease, excellent illustrations of which, as well as the original description, are afforded by Sieracki.[54] Section studies of perpiheral lymph nodes supplemented by the imprint method may now yield information of specific diagnostic importance in this condition and in Hurler's disease in which similar cells containing spindle particles have been recently described.[32]

SARCOMATOUS RETICULUM CELLS

Beyond reaction, however, is reticulum cell sarcoma. As large as the various malignant reticulum cells of this condition appear in sections, their



4

FIG. 19-4 A group of malignant reticulum cells in a lymph node imprint of reticulum cell sarcoma Leishman stain (× 1100)

bizarre, blastlike chromatin patterns, unusual nuclear configurations, and their nucleolar multiplications can only be truly appreciated with the more refined cytologic method seen in the lymph node imprints of reticulum cell sarcoma (Fig 19-4) In contrast to the reaction forms of the reticulum cell (Figs. 19-1, 19-2, 19-3), the cytoplasmic nuclear ratio now favors the hypertrophic malignant reticular nucleus The cytoplasm is scant, varyingly basophilic, and prone to vacuolar degeneration The leukemic manifestation of reticular malignancy of this type is usually classified in the literature as monocytic leukemia, and indeed differentiation to the monocytic line from the malignant reticulum cell does occasionally occur (Fig 19-10 and Table 19-2) and receives the eponymic designation of Schilling's type of

monocytic leukemia. The bizarre nuclear outlines apparent in some of the malignant reticulum cells of Figure 19-4 should not alone be misconstrued as diagnostic of monocytic differentiation. If one is to make the diagnosis of monocytic leukemia of the Schilling type, evidence for differentiation to true adult human monocytic forms (extreme right of Fig 19-10) should be established. Far more often the leukemic manifestation of reticulum cell malignancy, although similarly classified in much of the literature as



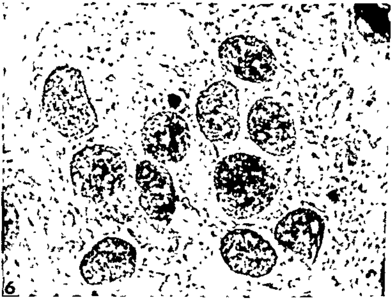
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FIG. 19 5 A lymph node imprint in Hodgkin's disease showing (*bottom*) a malignant reticulum cell, (*center*) a Reed Sternberg cell, and (top) a spindle-shaped endothelial cell Leishman stain (× 1100)

"monocytic" leukemic, is merely a leukemic outpouring of the malignant reticulum cells depicted in Figure 19-4 in considerable numbers into the patient's blood stream (Fig 19-9). Cases of this type in which the leukemic process is centered primarily in the malignant reticulum cells have been reported and correctly designated by Ewald,[20] Downey,[16] and others as leukemic reticuloendotheliosis With this background it is far more reasonable to appreciate the rare "monocytic" leukemia complicating Letterer-Siwe's disease or Hodgkin's disease as a similar accession of the malignant reticulum cells basic to these conditions to the blood stream

Characteristic of the imprinted lesions of the second reticular malignancy, Hodgkin's disease, are the giant, pleomorphic Reed-Sternberg cells, one of which is depicted in the center of Figure 19-5 All transitions are found between this giant cell peculiar to Hodgkin's disease and the atypical, large,

mononuclear reticulum cell depicted at the bottom of Figure 19-5. These background, malignant mononuclear cells bear a striking resemblance to the reticulum cells of reticulum cell sarcoma depicted in Figure 19-4 They are large in size and may present beginning lobulation of their nucleus. Their chromatin is arranged in small angular pieces but with distinct, intervening, colorless parachromatin spaces. The nucleoli, unlike their large, acidophilic counterparts in sectioned material, are small, round or irregular,



6

FIG 19-6 Imprint from supraclavicular lymph node showing metastatic tumor cells from Ewing's tumor in clavicle. Note resemblance to reticulum cells Leishman stain (× 1100)

and deeply basophilic. The cytoplasm is irregularly basophilic and moderately abundant. Binucleation of such cells leads to formation of the so-called mirror-image reticulum cells Lobulation of the nuclei of these malignant reticulum cells in Hodgkin's disease increases until the nucleus is giant, bizarre, and polymorphous (center of Fig. 19-5) It is to be noted that the fine reticular chromatin network with its chromatin-parachromatin distinction is retained in the fully developed Reed-Sternberg cell In imprints it will often be seen that each nuclear lobe is spotted with from six to eight small, basophilic nucleoli instead of the one or two large acidophilic nucleoli observed in histologic sections. However, even in imprints the nucleoli may reach diameters up to 7 μ, but they retain their deeply basophilic staining properties [48] The cytoplasm is abundant and consists of flaky or granular basophilic material against a colorless background

Megakaryocytes of comparable size and development will be found in the imprinted lesions of myeloid metaplasia and granulocytic leukemia. They may be distinguished readily from Reed-Sternberg cells by the heavy, coarse chromatin pattern possessed by the polymorphous, adult megakaryocytic nucleus Furthermore, the megakaryocytic cytoplasm presents a dense azurophilic granulation either spread diffusely throughout the cell body or in multiple focal aggregates prior to imminent platelet formation.[45] Finally, Fisher and Hazard[24] have shown that megakaryocytic cytoplasm is strongly positive for the periodic acid-Schiff method in contrast to the Reed-Sternberg cell.

The cell at the top of Figure 19-5 is an endothelial cell with its elliptical, finely stippled nucleus and spindle-shaped, elongated cell body.

Pleomorphic, giant multinucleated or polymorphous malignant reticulum cells are occasionally a feature of the lesions of reticulum cells sarcoma and frequently are present in imprints of lymph nodes in Letterer-Siwe's disease, the third member of this series (Table 19-1). Although suggestive of Reed-Sternberg cells, they never attain the complete characteristics so well described by Reed[49] in her 1902 paper, a paper which merits rereading.

A fourth member of this series is Ewing's tumor of bone. Figure 19-6 depicts the reticulum-like cells of this condition which were metastatic from the clavicle of a 11-year-old boy to a regional node and from there imprinted Ewing[21] suggested the "perivascular endothelium" as the cellular origin of his tumor. Oberling and Raileanu[41] and Stout[56] prefer to designate this tumor as a variant of reticulum cell sarcoma on the basis of the demonstration of reticulum cells as integral parts of them. Jaffe[31] agrees to these latter interpretations, sensibly suggesting that the name "Ewing's tumor" be retained, however, so that its distinction from primary reticulum cell sarcoma may be maintained.

METASTATIC TUMOR CELLS

Metastatic, anaplastic carcinoma cells present nuclei which may closely resemble malignant reticulum cells in imprinted preparations. This is another reason why the use of imprints is supplemental to the study of the histologic pattern in which the metastatic nature of the cell clusters is more often clearly identifiable. The reader will have no difficulty in making imprints of available tumors metastatic to regional nodes Sheets of monotonously patterned, anaplastic cells, unrelieved by the presence of any members of the lymphocytic series, extreme degrees of pleomorphism in clustered cell groups, and identifiable secretory or granular cytoplasmic products attest to the epithelial nature of metastatic tumor cells in imprints.

Metastatic nonreticular sarcoma cells may simulate reticular malignancy even more closely. In one such case that we have studied, the diagnosis of primary pleomorphic reticulum cell sarcoma was finally rejected only because of the demonstration of imprinted strap cells bearing the unmistakable cross-striations of the parent rhabdomyosarcoma

BLOOD-FORMING RETICULUM CELLS

In the foregoing we have described the cellular changes in some of the more important conditions affecting the reticulum cell as a reticulum cell or



7

Fig 19 7 Hematopoietic reticulum cell (*center*) from a lymph node imprint in infectious mononucleosis Compare with inactive reticulum cell of Fig 19-1 and reactive reticulum cell of Fig 19 2 Leishman stain (× 1100)

affecting the macrophage to which it is capable of transforming Heterologous lymphocyte formation by the reticulum cell (RE cell, mesenchymal cell, histiocyte) is also designated as *heterologous lymphocytopoiesis* It consists of a change *from the relatively inactive-appearing tissue RE cell* (Fig 19-11) to a free, active, blood-forming RE cell (Figs 19-7, 19-8) and only then to the lymphoblast (Fig 19-11) Most flow sheets derive the lymphoblast directly from the RE cell (stromal connective tissue cell), omitting reference to, and discussion of, some necessary intermediate (blood-forming RE cell) as the smaller, inactive RE cell nucleus becomes larger and changes from the coarser connective tissue nuclear pattern to the finer stipple or network

of the blast and as its extensive cytoplasm retracts freeing the cell and increasing its basophilia but depressing the cytoplasmic-nuclear ratio. In the study of hematopoiesis in the bone marrow after the fetal period and in the study of peripheral blood cells, such an oversight is understandable because nonleukemic, heterologous blood formation from reticulum cell through hematopoietic RE cell to the myeloid elements requires an almost cataclysmic stimulus (e.g., atomic radiation,[36] untreated subacute bacterial

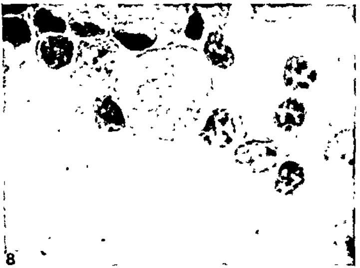


FIG. 19-8 Hematopoietic reticulum cell (*top center*) from a lymph node imprint in infectious mononucleosis This reticulum cell is well on its way to blood cell formation Leishman stain (× 1100)

endocarditis[10]) for its demonstration in those locations In contrast is the ease with which heterologous lymphocyte formation can be followed in lymph nodes responding to even moderate antigenic stimuli or to stimuli responsible for lymphocyte formation Imprints of any node reacting to such stimuli, and the majority of otherwise "normal" nodes are so reacting, will yield for study examples of all stages in the transformation of inactive reticulum cells first to hematopoietic reticulum cells and only then to lymphoblasts

In the transformation of the inactive or undifferentiated reticulum cell (Fig 19-1) to the cell capable of forming blood cells, the first change is the enlargement of the nucleus and an accentuation of the chromatin pieces forming the irregular network or stippled pattern with its now larger but still irregularly outlined, colorless parachromatin interstices (Fig 19-7) The cytoplasm then retracts and becomes increasingly basophilic (Fig 19-8),

the chromatin pieces become smaller and finer, and the resulting cell at this stage manifests its blood-cell-forming propensities. The poorly delimited, irregularly outlined nucleoli usually remain small in the nonleukemic transformations (Figs. 19-7, 19-8) but may become unduly prominent (Fig. 19-9) when the heterologous change is leukemic in nature. The hematopoietic reticulum cell has been descriptively labeled "hemohistiocyte" by Ferrata.[23]



9

FIG 19-9 Hematopoietic reticulum cells that were also leukemic Bone marrow of so called acute monocytic leukemia Leishman stain (× 1100)

LEUKEMIC BLOOD-FORMING RETICULUM CELLS

Ewald[20] first described a leukemic process centered at the hematopoietic RE cell stage, and Downey[16] followed with excellent descriptions of the blood-forming RE cell in leukemic and nonleukemic environments If the leukemic process consists of an overgrowth of leukemic hematopoietic RE cells without further differentiation toward any or the blood cell lines, then Ewald's and Downey's descriptive term "leukemic reticuloendotheliosis" applies, although the term "reticulum cell leukemia" is equally as descriptive and is more informative of the true nature of the condition than Aschoff's suggestion[1] of "histiocytic leukemia" The Italian designation of *hemohistioblastic leukemia* is a good descriptive term emphasizing that the leukemic process is centered between the tissue cell and the stem cells of the blood. Less fortunate is the term "monocytic leukemia" which has become

entrenched in the American literature as a designation for the leukemic overgrowth of blood-forming RE cells

In leukemias in which differentiation does not progress beyond the hematopoietic RE cells (Fig 19-9), the nucleus retains the reticulated or stippled pattern of its nonleukemic counterpart, the nucleus is larger than that of lymphoblasts or myeloblasts, and the cytoplasm is similarly much more abundant than the cell body of either the leukemic lymphoblast or myeloblast. Azurophilic granulation may be present or absent. Often the



FIG 19 10 Hematopoietic reticulum cells that were also leukemia but show all stages of transformation to true monocytes at far right Bone marrow of true acute monocytic leukemia, Schilling type Leishman stain (× 1100)

large cell body fails to round up and drags along a broad tail or cytoplasmic streamer Malignant lobulation of the nucleus can easily be misinterpreted as abortive attempts at monocyte formation (top cell of Fig 19 9). It is very rare for the cells of leukemic reticuloendotheliosis (histiocytic leukemia) to show differentiation to mature human monocytes (Fig 19-10) When such a rare case occurs, it is correctly designated as *monocytic leukemic reticuloendotheliosis* or *acute monocytic leukemia* (Schilling). More often, however, if the leukemic RE cells show some differentiation to recognizable blood cell lines, such differentiation is manifested by grotesque granulocyte formation on their part The resultant granulocytes are characterized by their large size, reticulum cell nuclear patterns, bizarre or malignant nuclear lobulation, but with their telltale, specific cytoplasmic

granulation, commonly neutrophilic, less frequently eosinophilic or basophilic. So common is this tendency of the leukemic RE cell to bizarre granulocyte formation that the nuclear lobulations mistakenly interpreted as "monocytic" in connotation are more often evidence of precocious granulocytic nuclear segmentation prior to specific granule formation. It has thus come about that in recent years granulocytic leukemic reticuloendotheliosis (i e., a monocytic leukemia with leukemic granulocytes) has been recognized under the term "myelomonocytic leukemia."



11

Fig 19 11 Central group of two lymphoblasts and a prolymphocyte as well as a medium sized lymphocyte in mitosis from a lymph node imprint in infectious mononucleosis Leishman stain (×1100)

Less common than granulocytic leukemic reticuloendotheliosis is the leukemic process in which the hematopoietic RE cells show some differentiation to leukemic erythroblasts, and thus erythrocytic leukemic reticuloendotheliosis is a frequent type among the rare group of the acute erythrocytic leukemias

It is interesting that lymphocytic leukemic reticuloendotheliosis, i e., a leukemia in which the hematopoietic RF cells show obvious differentiation to immature lymphocytes (lymphoblasts and prolymphocytes), is less common than its granulocytic counterpart (myelomonocytic leukemia) If such an admixture of hematopoietic RE cells and *immature lymphocytes is* infrequent in the leukemias, the opposite is the rule in the malignant lymphomas of a comparable degree of immaturity Thus, in malignant

lymphomas of the lymphoblastic cell type (Fig 19-12 and Table 19-1), it is rare indeed if hematopoietic RE cells of a malignant nature are not readily discernible in the imprints of affected nodes, spleen, or other organs involved.

LYMPHOBLASTS, NONLEUKEMIC

In reactive heterologous lymphocytopoiesis studied in imprinted material stained like blood smears, the resultant of the transformation of the inactive reticulum cell to a blood-forming reticulum cell is a nonleukemic lymphoblast The term "nonleukemic lymphoblast" is used here because the lymphoblast described and depicted in most textbooks of hematology is a leukemic lymphoblast as found in the blood of the acute lymphocytic leukemias The nonleukemic lymphoblast (left immature cell of Fig 19-11) of the lymphocyte-forming tissues at first shares in the abundant cytoplasm of its precursors, but it gradually presents only a moderate band of varying shades of pale to dark-blue color. Azurophilic granules are usually absent. The nuclear pattern is surprisingly similar to that of the blood-forming reticular cell nucleus with an irregular network of light-violet stipples or particles of chromatin sharply set off from colorless interstices, themselves irregular, angular, and unequal in size The nucleoli, which are always present, may be masked by the chromatin or as many as five nucleoli may be found, usually however, one or two pale-blue, round nucleoli are present The nuclear membrane is thin, thinner in fact than the nuclear membranes of the cells preceding or succeeding it in lymphocytopoiesis (Table 19-1) By reason of its larger size and reticular nuclear pattern, the lymphoblast of reactive lymphocytic tissues could be aptly designated a *reticular lymphoblast*

LYMPHOBLASTS, LEUKEMIC AND LYMPHOSARCOMATOUS

In contrast to the cell described above, the leukemic lymphoblasts of the acute lymphocytic leukemias are usually smaller and possess a nuclear pattern indistinguishable by ordinary staining methods from the myeloblast or stem cell of the marrow elements In this nuclear pattern, well illustrated in any textbook of hematology, the chromatin is arranged in fine strands or stipples with very regular but small interstices of colorless parachromatin in which are embedded none or from one to five pale-blue, round, or oval nucleoli It is paradoxical that the leukemic lymphoblast usually presents a neater, finer, more orderly chromatin arrangement than its normal counterpart

At times the lymphoblasts of lymphocytic leukemia structurally resemble

the reticular lymphoblasts as found in normal lymphocytopoiesis (Fig. 19-11), instead of resembling myeloblasts. This finding necessitates recognition of the possibility of encountering reticular lymphoblasts in an occasional case of lymphocytic leukemia in place of the lymphoblasts resembling myeloblasts in the ordinary case of acute lymphocytic leukemia. The former finding is especially true when hematopoietic RE cells are directly involved in the leukemic process together with the lymphoblasts (lymphocytic leukemic reticuloendotheliosis).

12



FIG 19-12 Lymphoblasts (*left*), lymphocyte (*center*), and prolymphocytes (*right*) in a lymph node imprint in lymphosarcoma, lymphoblastic cell type Leishman stain (× 1100)

In our experience the lymphoblasts of malignant lymphoma or lymphosarcoma of the lymphoblastic cell type (imprinted in Fig. 19-12) more closely resemble reticular lymphoblasts

We are now in a position to appreciate the additional fact that bizarre or malignant nuclear lobulation or indentation at times can be superimposed either on the leukemic lymphoblast with myeloblast-like nuclear pattern or on the reticular lymphoblast whether it is leukemic or lymphomatous Because of the foregoing, the leukemic lymphoblast presents itself in the peripheral blood with bizarre nuclear indentation or without it, with a myeloblast-like nuclear pattern or with one resembling a reticulum cell nucleus, heavily nucleolated, moderately nucleolated, or with masked nucleoli Rather than designate cells exhibiting one or the other of these unusual structural features as "*lymphosarcoma*" *lymphoblasts*, we prefer to

designate leukemic manifestations of malignant lymphomas as *leukemia* at that stage when the marrow or blood shows more than token evidence of involvement by the affected cell type.

PROLYMPHOCYTES

In homologous lymphocyte formation, lymphoblasts form additional lymphoblasts by mitosis or transform into immature lymphocytes intermediate

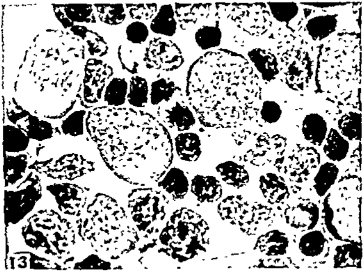


FIG 19 13. Three prolymphocytes (*top center*) surrounded by small and medium sized adult lymphocytes from a lymph node in infectious mononucleosis. Note the small lymphocyte (*center*) in mitosis Leishman stain (× 1100)

in differentiation between lymphoblasts and mature lymphocytes (Table 19-1). These intermediates are termed "prolymphocytes" (Figs 19-13, 19-14, 19-15). They may be the size of smaller lymphoblasts or may approach the size of the largest of the mature lymphocytes The cell body usually consists of a very narrow band although occasionally it is larger It possesses any degree of basophilia from pale to dark blue The most important distinguishing feature of the prolymphocyte is the finding in some nuclear areas of a fine network of chromatin strands still distinct from the colorless parachromatin interstices. Such immature areas blend imperceptibly with nuclear areas, *showing indistinct, coarse, clumped chromatin masses (Fig.* 19-13) In other words, the chromatin pattern of the prolymphocyte is too coarse to qualify as lymphoblastic and too fine and insufficiently massed to be designated as mature Occasionally a nucleolus is unmasked, but this

feature is of less diagnostic value than the immaturity of the nuclear pattern.

The finding of prolymphocytes as the dominant lymphosarcomatous type in a lymph node imprint of tissue histologically indicative of malignant lymphoma points to a cytologically subacute course. This is of practical importance because the lymphosarcoma cells in sectioned material may appear to be smaller and more mature than careful inspection of the same lesion imprinted will warrant. For example, sections of the giant follicular

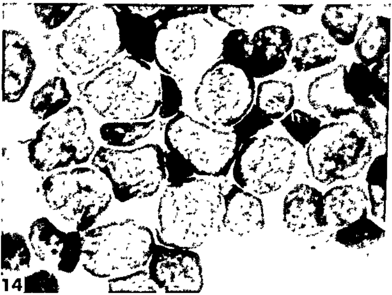


FIG 19-14 A large group of malignant prolymphocytes from a lymph node imprint in giant follicular lymphoma, prolymphocytic cell type Leishman stain (× 1100)

lymphoma imprinted in Figure 19-14 revealed that the nodules of the lymph node lesions were composesd of small, seemingly mature lymphocytes, suggesting a relatively favorable prognosis Study of the lymph node imprints, however, revealed a finer stippled chromatin pattern (Fig 19-14), diagnostic of immature lymphocytes (prolymphocytes), than had been anticipated, and a less favorable prognosis was indicated

Predominance of prolymphocytes in a lymphocytic leukemic picture (Fig 19-15) is diagnostic of a subacute lymphocytic leukemia Without specific therapy, subacute lymphocytic leukemia carries from six to twelve months survival time, whereas chronic lymphocytic leukemia affords a longer survival time, sometimes considerably longer Reference to Table 19-1 recalls another equally valid cellular distribution for a subacute lymphosarcomatous process or for the picture of subacute lymphocytic leukemia, and that is the

finding of a roughly equal admixture of lymphoblasts, prolymphocytes, and mature lymphocytes

MATURE LYMPHOCYTES

Prolymphocytes normally can form other prolymphocytes by mitosis (Table 19-1) or can transform into large, medium-sized, and small mature or adult lymphocytes (cf peripheral coarsely nucleated cells in Figs 19-1,

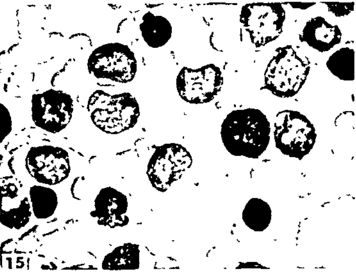


FIG 19 15 A group of leukemia prolymphocytes from the blood in a case of subacute lymphocytic leukemia Leishman stain (× 1100)

19-2, 19-18) Students of this field do not question the ability of the reticulum cell, lymphoblast, prolymphocyte, and large adult lymphocyte to undergo mitotic division That the medium-sized and small nonleukemic and nonmalignant lymphocytes are similarly capable of reproducing themselves has been disputed or denied It is now known that because of the unexpectedly long life span of the mature lymphocytes revealed by the radioisotopic investigations of Ottesen[42] and Hamilton,[27] mitotic figures among these cells are not numerous and do not need to be However, study of imprinted lymph nodes in nonmalignant conditions affords ample opportunity to demonstrate mitoses in medium-sized (Fig 19-11) and small, mature lymphocytes (Fig. 19-13 between the two central prolymphocytes)

In the lymphocytic tissues large, medium-sized, and small, mature or adult lymphocytes will be found Normally only the medium-sized and small, mature lymphocytes are present in the blood Size, however, is a relatively

feature is of less diagnostic value than the immaturity of the nuclear pattern.

The finding of prolymphocytes as the dominant lymphosarcomatous type in a lymph node imprint of tissue histologically indicative of malignant lymphoma points to a cytologically subacute course. This is of practical importance because the lymphosarcoma cells in sectioned material may appear to be smaller and more mature than careful inspection of the same lesion imprinted will warrant. For example, sections of the giant follicular



FIG. 19-14 A large group of malignant prolymphocytes from a lymph node imprint in giant follicular lymphoma, prolymphocytic cell type Leishman stain (× 1100)

lymphoma imprinted in Figure 19-14 revealed that the nodules of the lymph node lesions were composesd of small, seemingly mature lymphocytes, suggesting a relatively favorable prognosis. Study of the lymph node imprints, however, revealed a finer stippled chromatin pattern (Fig 19-14), diagnostic of immature lymphocytes (prolymphocytes), than had been anticipated, and a less favorable prognosis was indicated

Predominance of prolymphocytes in a lymphocytic leukemic picture (Fig 19-15) is diagnostic of a subacute lymphocytic leukemia Without specific therapy, subacute lymphocytic leukemia carries from six to twelve months survival time, whereas chronic lymphocytic leukemia affords a longer survival time, sometimes considerably longer Reference to Table 19-1 recalls another equally valid cellular distribution for a subacute lymphosarcomatous process or for the picture of subacute lymphocytic leukemia, and that is the

type, the chronic lymphocytic leukemias, and most of the lymphomas and leukemias complicated by Waldenström's syndrome. In diagnosis of the cell type in any of these conditions, attention is again directed chiefly to the nuclear chromatin pattern which is heavy and coarse and without evidence of immaturity. Nuclear and cytoplasmic vacuolation is not infrequent. The mature nuclear pattern in any of these conditions may be further complicated by bizarre nuclear indentations (Fig. 19-16), a complication which we have described in each succeeding member of heterologous and homologous



17.

Fig 19 17 A reticular plasmablast (*right*) and three proplasmacytes from bone marrow in multiple myeloma Leishman stain (× 1100)

lymphocyte formation. Even less frequent is the presentation of a large cell-bodied, atypical but mature leukocytoid lymphocyte (see below) as the predominant cell type of chronic lymphocytic leukemia. This unusual leukemia would seem to present almost insurmountable difficulties for differentiation from the reactive lymphocytosis of infectious mononucleosis if it were not for the fact that chronic lymphocytic leukemia is almost never found in children and young adults and is additionally lacking in the typical serologic results of the antibody (not absorbed by Forssman antigen but absorbed by bovine red cells) so prevalent in infectious mononucleosis.

LEUKOCYTOID OR ATYPICAL LYMPHOCYTES

These characteristically mature but atypical lymphocytes, unlike normal large lymphocytes which in spite of their greater diameter maintain the

unimportant feature in distinguishing these cells from immature lymphocytes Attention must be paid to the nuclear pattern in which large, coarse, dark-purple chromatin blocks blend imperceptibly with the colorless or light-blue parachromatin (periphery of Figs. 19-1, 19-2, 19-18). There is little or no chromatin-parachromatin distinction, although larger amounts of parachromatin are present in the nuclei of the larger mature lymphocytes Nucleoli are completely masked, but too much pressure applied in imprint-



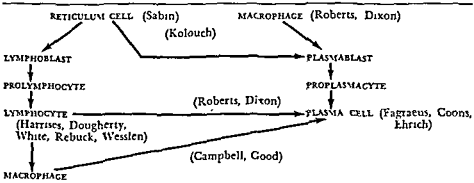
FIG 19-16 Leukemic adult lymphocytes from the blood in a case of chronic lymphocytic leukemia Note bizarre nuclear indentations which may connote malignancy but do not identify degree of differentiation since similar bizarre nuclear configurations are present in the reticulum cells of Fig 19-4 and the prolymphocytes of Figs 19-14 and 19-15 Leishman stain (× 1100)

ing lymphocytic tissues or in making blood smears will readily unmask the nucleoli present in even mature lymphocytes The nuclear membrane is thick and heavy Normally, even in the larger adult forms, there is only a narrow or moderate band of pale to dark-blue cytoplasm Large amounts of cytoplasm immediately place the mature cells into the class of atypical lymphocytes (see below) Round, red, relatively large, but sparse azurophilic granules may or may not be present. Normal, mature lymphocytes may present some nuclear indentation, although extreme degrees of nuclear lobulation suggest atypism or even malignancy

Malignancies of mature lymphocytes include diffuse lymphosarcoma of the lymphocytic type, giant follicular lymphoma of the lymphocytic cell

that they serve to recapitulate current concepts of heterologous and homologous lymphocyte formation (Table 19-1). Sabin[52] presented evidence for the participation of the reticulum cell in antibody formation Just a little earlier Kolouch[34, 35] associated heterologous plasmacytopoiesis from the reticulum cell with antibody formation responding to repeated antigenic stimuli Fagraeus,[24] Coons[8] and Ehrich[19] further established the close association of homologous plasmacytopoiesis with antibody production, usually after repeated antigenic stimulation Dougherty, White, and their associates,[13, 15] the Harrises,[28, 29, 47] and Wesslen[61] have demonstrated homologous lymphocytopoiesis and the lymphocyte as a cellular source of antibodies usually

Table 19 5. Antibody Formation



in response to a single antigenic stimulus Roberts, Dixon, and Weigle[51] have shown further that transferred lymphocytes are capable of antibody formation but transform into plasma cells in the process Finally, Campbell and Good[5] and Roberts and his associates[51] have similarly demonstrated the transformation of sessile macrophages (histiocytes) into plasma cells concurrent with their production of antibodies Had this information been available to earlier workers, it would have been apparent that multiple myeloma is but a malignant caricature of heterologous plasmacytopoiesis in which the reticulum cell forms malignant reticular plasmablasts and proplasmacytes diagnostic of this condition (Fig 19 17) Inherent abnormalities of gloubulin formation are thus warranted Similarly, in our experience Waldenstrom's syndrome complicating lymphocytic malignancies of the mature lymphocytic cell type is explained by retention on the part of the leukemic or malignant adult lymphocyte of its propensity for plasma cell formation (Fig 19-18) resulting in excess globulin formation even though in such cases the globulin formed is a giant polymer

low cytoplasmic-nuclear ratio of the small mature lymphocytes, have an overabundance of cytoplasm as their most outstanding feature (cf. Chapter 11) The increased cytoplasm may be pale in color, deeply blue, or a mixture of the two shades. The basophilic material may radiate in spokelike fashion through the paler cell body. It may or may not contain round, purple azurophilic granules Such granules, if present, may be small or large sparse or numerous. Vacuoles may be present. The nuclear pattern may be

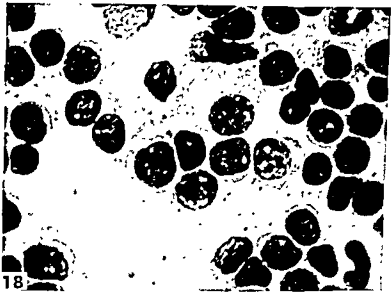


FIG 19 18 A central group of plasma cells All stages of transformation from the surrounding lymphocytes to plasma cells are present Bone marrow showing chronic lymphocytic leukemia complicated by Waldenstrom's syndrome Leishman stain (× 1100)

identical to that of the normal lymphocyte, or the pattern may be coarser, if possible, with larger chromatin blocks apparent. Vacuoles may be present in the nucleus The nuclear membrane is thick, and the nucleus is eccentrically positioned

A small percentage of such atypical or leukocytoid lymphocytes may be present in normal individuals,[45] increasing percentages, however, are characteristic of (but not diagnostic of) infectious mononucleosis,[17] viral hepatitis,[37] adrenal hypocorticism,[14] and in rare instances chronic lymphocytic leukemia as described above

CYTOLOGY OF ANTIBODY FORMATION

If the experimentally documented concepts of the cellular lineages involved in antibody formation are outlined (Table 19-3), it will be found

protein secretions Protein or glycoprotein secretions may be precipitated as intracytoplasmic colorless or red crystalloids, or they may be present as pale or dark-blue globules or rarely as acidophilic round structures Such globular inclusions, regardless of their staining reactions in imprints or marrow smears, probably correspond to the acidophilic Russell bodies in tissue sections The finding of red-staining cytoplasmic buds at the periphery of the cell body has elicited the descriptive term "flaming plasma cell "

Malignant structural features comprise the customary cellular signs of this change and include nuclear and nucleolar gigantism, bizarre nuclear lobulations, and abnormal mitoses The type of secretion is not helpful in this respect when examined within the cells themselves by ordinary methods. Of more importance for the diagnosis of a malignant plasmacytosis is the finding of predominant plasmablasts and proplasmacytes (Fig 19-17) with disproportionately small numbers of adult plasma cells. In contrast, the reactive plasmacytoses of antibody formation (e g., infection, amyloidosis, rheumatoid arthritis, disseminated lupus erythematosus) are characterized by the presence of numerous adult Marschalko type plasma cells in the company of only small percentages of their immature precursors. It is in such population analyses that imprint studies show their greatest value.

LYMPHOCYTE FORMATION OF PLASMA CELLS

The formation of plasma cells by lymphocytes (Fig. 19-18) appears to be accomplished by gradual hypertrophy of the narrow cell body. As the cytoplasm increases, the basophilia deepens The nucleus becomes eccentric, and *the diffusely coarsened chromatin aggregates into blocks Concurrently the* nonstaining cellular organelles mass into a focal area about the cell center to form an irregularly clear area known as the *hof*. All transitional stages may be seen in the central cells of Figure 19-18 Experimental evidence in animals demonstrates that lymphocytes are undoubtedly capable of forming plasma cells, but in doing so they first formed macrophages in Campbell and Good's work[5] and "transitional" cells in Roberts' experiments (Chapter 7). Obviously further studies of imprinted reactive plasmacytoses within the lymphocytic tissues of man together with simultaneous plasma and cellular protein analyses are indicated

SUMMARY

Cellular descriptions of each of the members of the lymphocytic and plasmacytic series have been given as they appear in imprinted preparations of the lymphocytic tissues, marrow aspirates, and blood smears These comprise the inactive reticulum cell, the blood-forming reticulum cell and blasts, and intermediates and resulting adult cells which are formed

RETICULUM CELL FORMATION OF PLASMA CELLS

The major structural changes in the transformation of the reticulum cell to the (reticular) plasmablast are noted in the cytoplasm (Fig. 19-17, upper right). The customarily pale cytoplasm of the inactive reticulum cell becomes more basophilic at first near the enlarging cytocentric region and then gradually throughout the entire cell body. The cytoplasmic organelles, which do not stain with ordinary blood stains, show a beginning tendency to aggregate in the region of the cytocentrum which emerges as an indistinct and paler area (hof) in close association with the eccentrically placed nucleus Azurophilic granules are exceedingly rare. Evidence of peripheral cytoplasmic dissolution is commonplace. The round or oval eccentrically placed nucleus has a thin nuclear membrane and a chromatin pattern of fine chromatin strands arranged in an irregular network with distinct pink or yellow parachromatin interstices. The nuclear pattern closely resembles that of the inactive reticulum cell nucleus (Fig 19-1) but has thicker chromatin strands; none or from one to six blue nucleoli may be present.

An intermediate stage, the proplasmacyte, intervenes between the reticular plasmablast and the mature plasma cell. The proplasmacyte (Fig. 19-17, left and top center) has abundant cytoplasm but usually less than the plasmablast With shrinkage of the cytoplasm, its basophilia becomes more prominent Aggregation of cytoplasmic organelles in the region of the cytocentrum can result in a clearer perinuclear area (hof) than in the plasmablast Peripheral cytoplasmic dissolution (cytoplasmic shedding) may still be prominent The round or oval nucleus remains small and eccentric The nuclear membrane is thickening The chromatin pattern shows coarsening and thickening of the reticular network, however, irregular interstices of parachromatin are still distinct. One or two nucleoli may still be visible although they may be masked

With the transformation to the adult or Marschalko type plasma cell completed, the cytoplasmic-nuclear ratio still favors the cytoplasm in many cells. although the cytoplasm is less abundant than in the proplasmacyte. Typically the deep-blue cytoplasm has a large, pale-staining perinuclear area, the hof Peripheral *cytoplasmic dissolution may persist but is less prominent* The eccentric nucleus possesses a thick nuclear membrane The chromatin shows further condensation The coarse chromatin masses are arranged in irregular clumps and tend to pyknosis The parachromatin is small in amount but distinct in the few remaining interstices of the chromatin until pyknosis supervenes. Nucleoli are no longer visible

Nonmalignant variations of the plasma cell comprise nuclear budding, orderly binucleation or multinucleation, vacuolation, and various types of

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INDEX